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FORMATION OF INTERCELLULAR SUBSTANCE BY THE ADMINISTRATION OF ASCORBIC ACID (VITAMIN C) IN EXPERIMENTAL SCORBUTUS *

VALY MENKIN, M.D., S. BURT WOLBACH, M.D., AND MIRIAM F. MENKIN, M.A.

(From the Department of Pathology, Harvard University Medical School, Boston, Mass.)

In 1919 Aschoff and Koch [1] expressed the belief that in scurvy the primary deficiency consisted of a lack or faulty development of cement substance. They inferred that the condition at the costochondral junction in human scorbutic material was due to the inability of osteoblasts to form osteoid tissue. The experiments of Wolbach and Howe [2] demonstrated without any doubt that the immediate effect of orange juice administration in the repair of scorbutus is prompt deposition of intercellular material. This reparative process was clearly shown in several ways, notably by the renewed formation of dentine in the incisor teeth of guinea pigs, the deposition of a homogeneous matrix by the periosteal layer of cells and, finally, by the formation of osteoid and osseous trabeculae in the *Gerüstmark* of the costochondral junctions. More recently Wolbach [3] demonstrated that the deposition of collagen in the organization of blood clots in the state of absolute scorbutus was referable to the administration of vitamin C and that it represented the product of fibroblastic secretory activity. The earlier studies of Wolbach and Howe led these investigators to consider scorbutus as a state characterized primarily by a cessation in the normal formation of intercellular substance on the part of supporting tissues.

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The object of this brief report is to record the histological response produced by the administration of crystalline ascorbic acid (vitamin C) in the amelioration of experimental scorbutus in guinea pigs. That ascorbic acid prevents the development of scorbutus in guinea pigs and cures the condition when established has been proved. The observations that are to follow show that the histological repair is identical with that produced by vitamin C in orange juice.

Advances toward the isolation of vitamin C in its crystalline form have been rapid since 1928 when Szent-Györgyi isolated a reducing substance from various plants and subsequently from adrenal cortex which he called *hexuronic acid*.[4] From the very beginning Szent-Györgyi recognized the similarity of this acid to vitamin C. In the early part of 1932, in collaboration with Svirbely, this investigator reported that a daily allowance of 1 mg. of hexuronic acid afforded complete protection against scurvy in a 90 day test.[5] According to Szent-Györgyi this substance is a monocarboxy acid corresponding to the formula $C_6H_8O_6$. More recent studies in association with Haworth [6] have shown that it has evidently no relation to uronic acid and that it is most likely a lactone. Szent-Györgyi proposed the name of *ascorbic acid* for the crystalline product. Independently, and practically simultaneously with the isolation of ascorbic acid by Szent-Györgyi, King and his collaborators obtained crystals from lemon juice that had identical effects on scorbutic animals.[7] Recently Reichstein and his collaborators [8] succeeded in synthesizing a product from l-xylosone having properties that they believe are identical with those of natural ascorbic acid.

The crystalline vitamin C preparation, as isolated and used in the experiments that are to be reported here, was obtained from lemon juice by modifying only slightly the original methods of extraction, as reported in the various communications of King and his co-workers.[9-12] The reader is referred to these publications for details of the chemical technique. There is, however, one point that perhaps deserves mentioning. In the final step of extraction crystallization of ascorbic acid was found more effective when the material was maintained at minus 20° C for several days. Furthermore, its potency seems to be distinctly decreased when the various steps in the isolation process are carried out in the presence of oxygen. One such preparation, when administered to scorbutic guinea pigs, yielded definite reparative response in the incisor teeth but failed to amelio-

rate the condition in the *Gerüstmark* of the costochondral junction. This would indicate that the reparative response to antiscorbutics is more sensitive in the incisor teeth than at the costochondral junction. A second preparation in which adequate precautions were taken to isolate the material in a relatively oxygen-free atmosphere by bubbling CO_2 during the various evaporations *in vacuo* yielded a product sufficiently potent so that adequate repair could be demonstrated both at the costochondral junction and in the incisor teeth.*

The diet employed was the same that Wolbach and Howe had used in 1926. The guinea pigs had an initial weight ranging between 250 and 400 gm. The ascorbic acid solution was administered either orally or parenterally, 3 to 5 mg. per day as a rule. The treatment was started 2 to 3 weeks following the onset of the scorbutic diet, at a time when the symptoms of the disease were conspicuous. The treatment with ascorbic acid was continued for a period varying from 2 to 15 days, after which the animal was chloroformed and a thorough postmortem examination performed.

The histological studies were centered chiefly in following the reparative processes occurring in sections of the costochondral junctions and through the incisor teeth. Wolbach and Howe in their original study found that these structures gave them exceedingly good material for studying scorbutic sequences. In brief, they have pointed out that the important change in the incisors of scorbutic guinea pigs is cessation of formation of dentine and separation of the odontoblast layer from the dentine (Fig. 1). In complete scorbutus the presence of liquid separating the odontoblasts from the dentine seems to be the result of a defective secretion of the odontoblasts. Furthermore, they showed that the administration of orange juice is rapidly followed by the deposition of newly formed dentine in the irregular contours of the odontoblast layer. On the basis of their findings they expressed the view that "the missing factor or agent which the antiscorbutic enables the odontoblasts to supply is evidently one effecting the jelling or setting." At the costochondral junction of scorbutic animals they found that osteoblastic proliferation continues uninterruptedly without, however, producing any bone matrix. These cells assume the shape of fibroblasts, and the

* Professor Szent-Györgyi was kind enough to send us a sample of ascorbic acid which we have also been able to test on some of our scorbutic animals. The histological findings were similar to those obtained with the product isolated in this laboratory.

resulting edematous appearance of the loosely textured connective tissue has been given the name *Gerüstmark*. The initial administration of orange juice is promptly followed by a diffuse deposition of osteoid matrix. On further treatment with the antiscorbutic Wolbach and Howe described the formation of definite osteoid and osseous trabeculae replacing the loosely textured *Gerüstmark*. Here again, as in the case of the odontoblasts, they interpret the activity of the osteoblasts in scorbutus as that of cells producing a defective liquid product.

Results

The data obtained following the administration of ascorbic acid to scorbutic guinea pigs were exactly in accord with the previous findings of Wolbach and Howe with orange juice. This would seem to prove that the deposition of intercellular substance subsequent to the feeding of orange juice is due to vitamin C *per se* and not to some other ingredient. Most of the animals showed distinct symptomatic improvement soon after the ascorbic acid feeding was initiated, as indicated by both increased weight and restoration of appetite.

The incisors of scorbutic guinea pigs receiving no ascorbic acid showed, as stated above, the characteristic separation of dentine from the odontoblast layer and a somewhat shrunken pulp (Fig. 1). Scorbutic guinea pigs given the crystalline product displayed marked deposition of dentine precisely as had been previously found with orange juice (Fig. 2). Furthermore, the amount of intercellular dentine laid down follows roughly the total amount of ascorbic acid administered. Parenteral injection of the crystalline product is at least as effective in producing reparative reactions as oral administration.

Sections through the costochondral junction of scorbutic animals show the typical loosely textured *Gerüstmark* containing fibroblast-like cells producing no osteoid matrix. There is considerable hemorrhage; the periosteum is thinned out with cell bodies in close apposition to each other and devoid of the normal homogeneous matrix (Fig. 3). By contrast Figure 4 illustrates the same region in a scorbutic guinea pig that received one intraperitoneal and three daily subcutaneous injections each of 5 mg. of ascorbic acid. The site of parenteral injection was shifted from day to day so as to obviate any retardation in absorption by the possible development of an inflammatory reaction sufficiently severe to obstruct lymphatic drainage.[13]

The *Gerüstmark* is interspersed with osteoid trabeculae extending from the columns of cartilage to the marrow. The periphery of these trabeculae is lined by numerous osteoblasts. The repair is complete, involving also cartilage and periosteum (Fig. 5). Adjacent to the cartilage columns, cells are seen that form either osteoid matrix, chondromucin or the homogeneous collagenous matrix of periosteal tissue (Fig. 5). In a guinea pig sacrificed after receiving a total of 75 mg. of the crystalline material orally over a period of 15 days the newly formed osteoid trabeculae at the *Gerüstmark* were found ossified, with evidence of progressive bone resorption (Fig. 6).

Discussion

The observations cited above show that the administration of the crystalline material known as *ascorbic acid* to scorbutic guinea pigs induces reparative reactions identical in character with those obtained with orange juice. The cessation in the formation of intercellular substance by supporting tissues in experimental scorbutus is therefore doubtless due to the lack of vitamin C, which is ascorbic acid. It is interesting to note that a function of cells as important as the production of intercellular matrix seems to depend on the presence of a relatively simple chemical substance, having as its empirical formula $C_6H_8O_6$. The mechanism of this reaction still remains to be elucidated. Further studies are being conducted in an endeavor to clarify this problem. The observations of Szent-Györgyi suggest the intimate rôle that ascorbic acid may play in cellular oxidation [14] in connection with its unusual reactivity as a reducing agent. This investigator advances the theory that ascorbic acid plays a central rôle in cellular reactions by acting as a regulator of potential changes, in this way preserving protoplasm against oxidation. He showed that silver salts, even in acid solution, are immediately reduced by ascorbic acid, a capacity unparalleled by substances composed solely of carbon, oxygen and hydrogen. While acting as a reducing agent ascorbic acid oxidizes itself, this being a reversible phenomenon. Szent-Györgyi expresses the belief that the biological function of ascorbic acid resides in its oxidation-reduction properties, in that it acts as a sort of buffer which links the various oxidizing enzymes participating in cellular metabolism. He has obtained some support for this view in studies on the respiration of the cabbage leaf.[15] The

oxidation-reduction potential of ascorbic acid has also recently been studied by Fruton [16] and others. Evidences of ascorbic acid acting as an activator of enzyme action have also recently been brought forward by Purr,[17] and Karrer and Zehender.[18] Harrison [19] has lately studied the oxygen consumption of tissues removed from scorbutic animals and has shown that the uptake is considerably lower than that from normal animals. The addition of small amounts of ascorbic acid to such tissues *in vitro* brings about an increase in the oxygen consumption. In the case of normal tissues, however, the oxygen uptake remains unaffected by the addition of ascorbic acid. It is conceivable, in view of such observations, that the lack of intercellular substance in the supporting tissues of scorbutic animals, as evidenced histologically, may be an expression of reduced cellular oxidation resulting from a deprivation of ascorbic acid.

Summary and Conclusions

Ascorbic acid (vitamin C in crystalline form) administered orally or parenterally to scorbutic guinea pigs induces reparative processes, as demonstrated by the renewal of dentine formation in the incisor teeth and by the deposition of osteoid matrix and chondromucin at the costochondral junction. These results on the formation of intercellular substance are in accord with those previously obtained by Wolbach and Howe by feeding orange juice. They furnish additional support for the view that ascorbic acid is indistinguishable from vitamin C. The evidence obtained indicates that a relatively simple chemical substance, ascorbic acid, controls the deposition of intercellular substance. The possible mechanism involved in this reaction is discussed from the standpoint of the properties of ascorbic acid as a reducing agent in relation to cellular oxidations.

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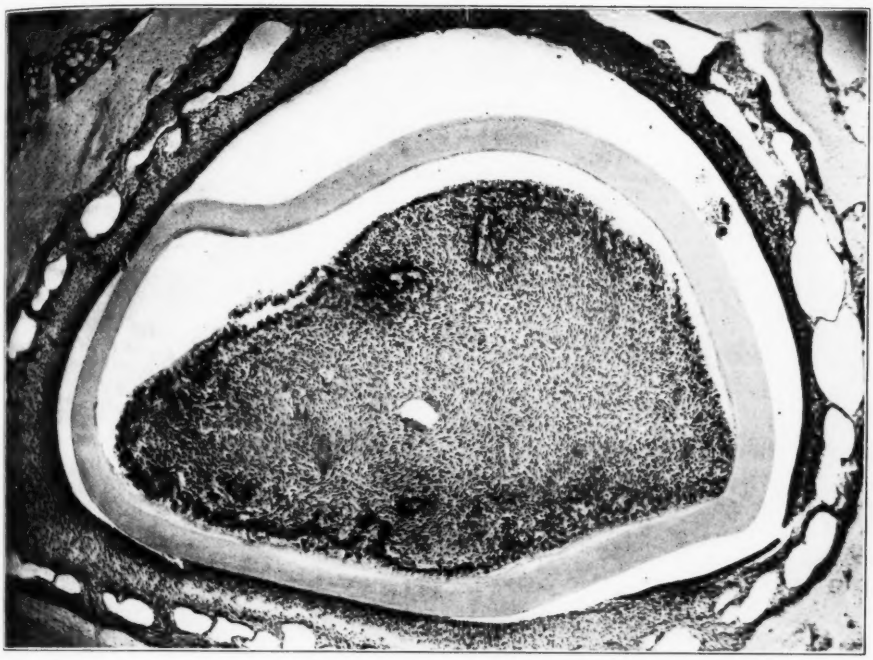
DESCRIPTION OF PLATES

PLATE 135

FIG. 1. Section through incisor of a scorbutic guinea pig. The separation between the odontoblast layer and the dentine is striking. Low power.

FIG. 2. Section through incisor of experimental scorbutic guinea pig. 20 mg. of ascorbic acid administered parenterally over a period of 4 days. The animal was then killed. Note the large amount of newly formed dentine. Low power.



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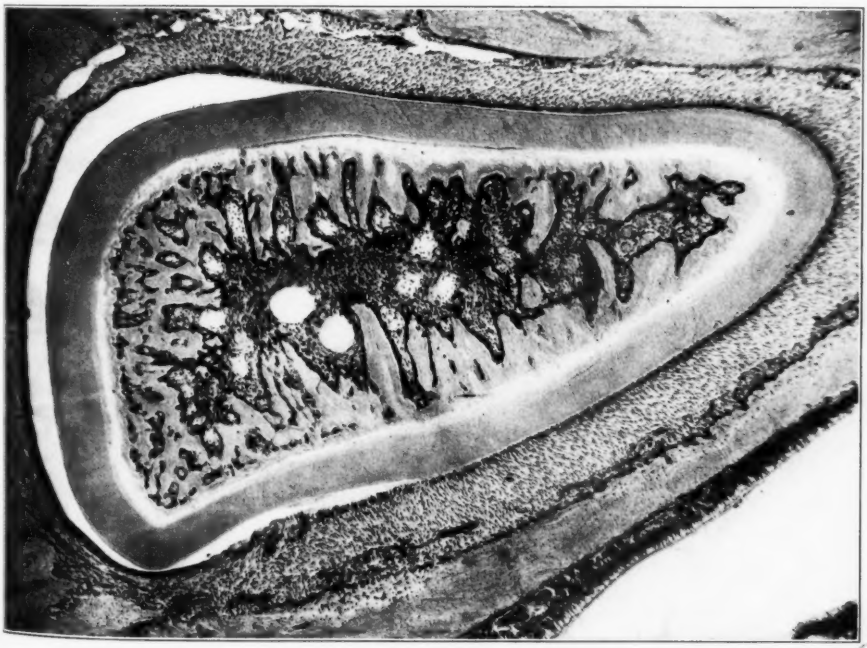


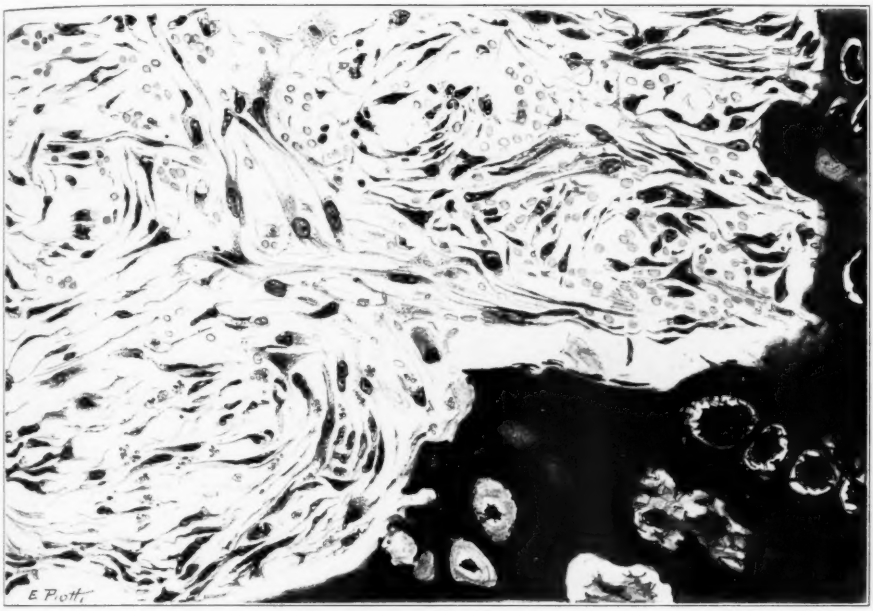
2

Menkin, Wolbach and Menkin Formation of Intercellular Substance

PLATE 136

FIG. 3. High power drawing through costochondral junction of scorbutic guinea pig, showing the loosely textured *Gerüstmark*. There is some evidence of hemorrhage. Since the cells have failed to produce any intercellular substance it is practically impossible to differentiate osteoblasts from fibroblasts.

FIG. 4. Section through costochondral junction of experimental scorbutic guinea pig that had received four parenteral injections of ascorbic acid aggregating to 20 mg. Note at the *Gerüstmark* the extensive repair reaction in the form of newly deposited osteoid trabeculae. × 175.



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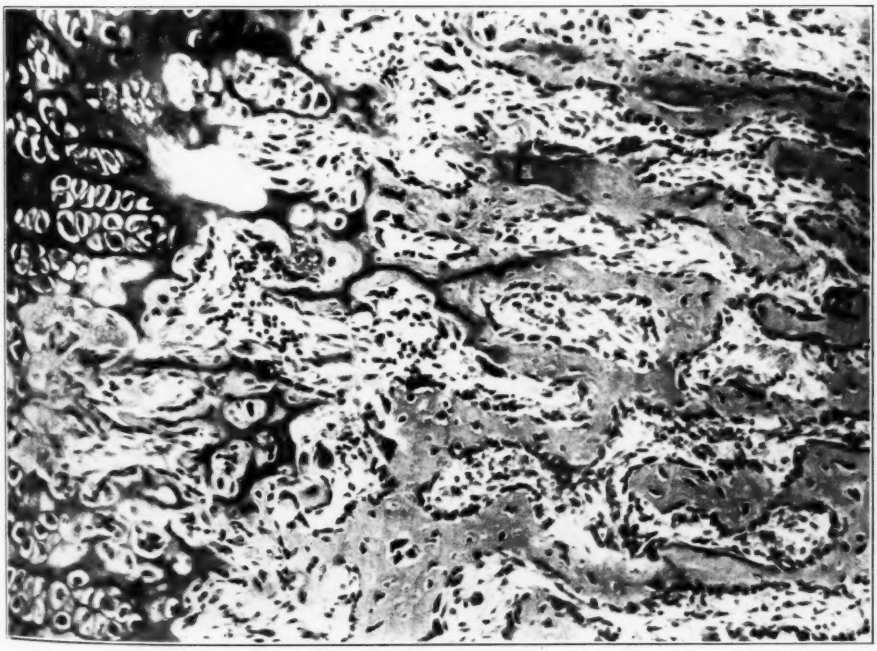

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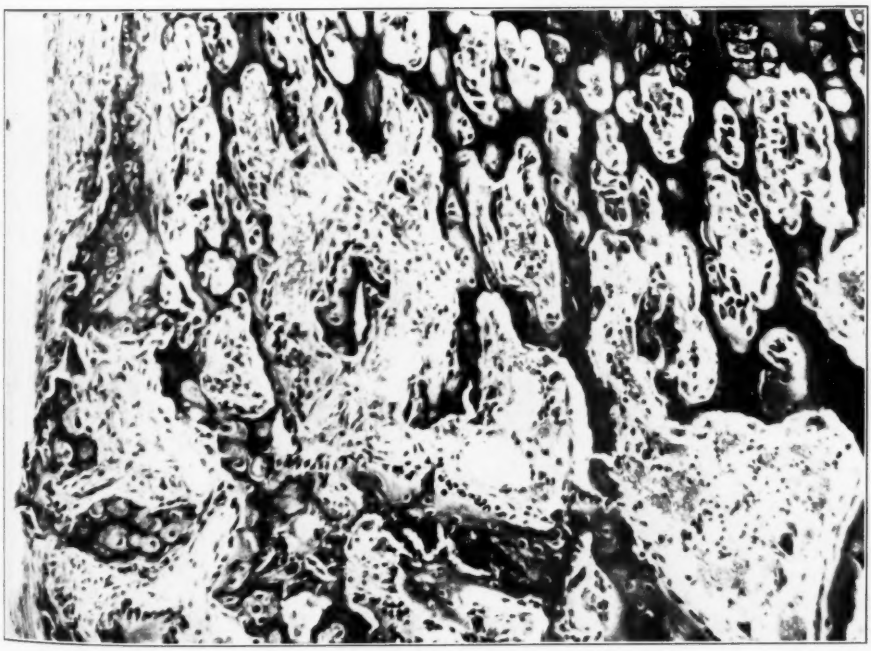
PLATE 137

FIG. 5. Same guinea pig as Fig. 4, showing reparative reaction of cartilage and periosteum at the costochondral junction following four parenteral injections of ascorbic acid. About × 160.

FIG. 6. Costochondral junction of experimental guinea pig that had received orally 75 mg. of ascorbic acid over a period of 15 days. Note the osseous trabeculae with progressive bone resorption. Compare with deposition of osteoid matrix in Fig. 4 following only 4 days treatment with ascorbic acid. × 175.

5



6

INCLUSIONS IN RENAL EPITHELIAL CELLS FOLLOWING THE USE OF CERTAIN BISMUTH PREPARATIONS *

ALWIN M. PAPPENHEIMER, M.D., AND EUGENIA H. MAECHLING, PH.D.

(From the Departments of Pathology and Dermatology, College of Physicians and Surgeons, Columbia University, New York City)

It is not the purpose of this paper to discuss the pathology of bismuth nephritis in general, but rather to call attention to peculiar epithelial inclusions, which seem to be definitely related to the administration of certain bismuth compounds. The fact that these bodies are found within the nucleus as well as in the cytoplasm lends them particular interest and justifies a detailed description. The bodies were first observed in Case 1. A second case, in which a different bismuth preparation was used, and which also showed similar inclusions in the renal epithelium, is also reported.

CASE REPORTS

CASE 1. P. H. History No. 357,602. The patient, E. R., a male, 46 years of age, was admitted to the Presbyterian Hospital on Oct. 28, 1932, and again on Dec. 9, 1932. The chief complaint was epigastric pain and loss of weight for 5 months. The family history was unimportant. The patient had been healthy and robust until the onset of the present symptoms. There was a history of gonorrhea 23, 20 and 9 years ago, but no syphilitic infection. The Wassermann reaction was reported negative in 1923, 9 years previous to the present illness.

Five months ago the patient began to have pain and a feeling of fullness in the epigastrium. The pains were gnawing in character, occurred 1 to 2 hours after meals and occasionally at night. The pain was relieved by vomiting. Over a period of 3 months there was a loss of 16 pounds in weight. He entered the hospital in October, remaining for 3 weeks. Physical examination at that time disclosed a palpable mass in the epigastrium. The Wassermann reaction was 4 plus, and because of the possibility that the mass was a gumma of the liver he was referred to the Dermatological Clinic for antiluetic treatment. On November 22nd, and again on the 25th, 29th and on December 2nd he received 2 cc. of bismocymol (0.1 gm. bismuth) intramuscularly. In addition, he was given three intravenous injections of Old Salvarsan, totalling 0.6 gm., and 1 gr. of mercuric salicylate intramuscularly.

There was no decrease in the size of the abdominal mass under treatment. The weakness and loss of weight progressed, and the patient was readmitted to the surgical service.

Physical Examination: The temperature was 98.4, pulse 80, respiration 20, blood pressure 115/90. The head and thorax were normal. In the epigastrium

* Received for publication November 2, 1933.

a large, hard, irregular mass could be felt with the lightest touch and its outlines could be seen through the abdominal wall. It extended two-thirds of the way to the umbilicus and was slightly tender. Cervical, axillary and right epitrochlear glands were definitely large but not tender. Examination otherwise negative.

Laboratory Findings: Hemoglobin 75 per cent, red blood cell count 4,150,000. Wassermann reaction 4 plus. Stool guaiac-negative. X-ray showed a broad crater shadow along the lesser curvature of the stomach. Urinalysis showed a trace of albumin, many hyaline and granular casts and occasional white blood cells.

An exploratory laparotomy was performed 3 days after admission and an extensive carcinomatous infiltration of the liver was found. This was confirmed by examination of an excised specimen. Following the operation a hematoma occurred in the lower third of the wound. The patient became increasingly weak and died on the 12th day after operation.

Clinical Diagnoses: Carcinoma of stomach with metastases to liver; syphilis.

Postmortem Examination

Autopsy No. 11,124, performed 16 hours after death.

Anatomical Diagnoses: Gastric ulcer and carcinoma, metastases in regional lymph nodes, liver and lung; syphilitic aortitis, syphilitic orchitis with gummas; atherosclerosis.

The stomach was the seat of a carcinomatous ulcer 2.5 by 3 cm., situated on the lesser curvature 4 cm. from the pylorus. Nodules of tumor tissue were seen in the base of the ulcer and in the lesser omentum. The liver weighed 5350 gm., was greatly enlarged and filled with carcinomatous nodules. Normal liver tissue was found only in one small area in the right lobe. The aorta presented gross and microscopic lesions of syphilitic aortitis. Both testes were fibrotic and contained gummatous areas of caseous necrosis.

Kidneys: No gross changes were visible. The right kidney weighed 140 gm., the capsule was easily removed, the surface smooth and brownish. The cortex was 7 mm. wide. The markings were regular and distinct. There was no gross vascular thickening. Pelves and ureters were normal. The left kidney was similar in appearance.

Microscopic Examination of Kidneys

Microscopically no significant changes are found in the glomeruli. The capillaries contain a moderate amount of blood, the basement membrane is not thickened and there is no increased cellular content. A small amount of granular coagulum is seen in some of the capsular spaces.

Interesting changes, however, are found in the epithelial cells of the convoluted tubules, and chiefly in the distal portions. The lining cells are atypical, often flattened or irregular in shape; the nuclei are variable in size and occasional mitotic figures are seen. Often the lumen is filled with exfoliated necrotic cells or cellular detritus which stains deeply with eosin. In some tubules one has the impression of a coagulative necrosis affecting the portion of the cell contiguous to the lumen, but leaving the basal portion of the cell still viable.

Peculiar bodies are found in many of these atypical or degenerating epithelial cells. They are both cytoplasmic and intranuclear. Their size varies from 2 to 5 microns, averaging perhaps half the size of a red blood corpuscle. Their shape is usually spherical, although elliptical and obovate forms are occasionally seen. They have, in unstained formalin-fixed frozen sections, a slightly brownish color, and are highly refractile with a dark, singly contoured membrane. In some tubules they are quite numerous; others contain only one or two.

Intranuclear bodies are usually surrounded by a pale halo (Fig. 1). The nucleus containing the inclusion is often slightly enlarged or hydropic and the nucleolus displaced to one side against the nuclear membrane. One nucleus is found in which the slightly elongated inclusion has apparently bulged out the nuclear membrane preparatory to escaping into the cytoplasm (Fig. 2).

The cytoplasmic bodies are usually located between the nucleus and free border, or at one side of the nucleus (Figs. 3 and 4). Frequently they are found within the necrotic desquamated cells within the lumen. In general, their presence is associated with, possibly leads to, necrosis of the containing cell. A characteristic inclusion, however, is found within a cell undergoing mitosis, and many of the cells with intranuclear bodies show little if any degenerative change. This suggests that the inclusions are first formed within the nucleus, to be later extruded as the cell degenerates.

CASE 2. P. H. History No. 405,156. The patient, a female, 60 years of age, was first admitted to the Neurological Institute on Dec. 11, 1933. For 3 years she had suffered from generalized headaches, attacks of flushing and dizziness. A year ago she had a slight stroke, with speech impairment and right hemiparesis. A second similar attack occurred in November, 1933.

On physical examination right hemiparesis, adiadokocinesis, rambling speech and exaggeration of reflexes on the right side were present.

Laboratory Findings: Red blood cells 4,300,000, white blood cells 7500, polymorphonuclears 70 per cent, hemoglobin 78 per cent. Blood Wassermann reaction 4 plus. Spinal fluid 0, gold curve 1111000000, Wassermann negative. Urine showed a faint trace of albumin and clumps of pus cells.

A diagnosis of meningovascular syphilis with thrombosis of left anterior and middle cerebral arteries was made. The patient was given a course of antisyphilitic treatment. She received 10 intramuscular injections of potassium bismuth tartrate with butyn (Abbott Laboratories) over a period of 31 days, beginning December 19th. The total amount administered was the equivalent of 1 gm. of metallic bismuth.

On January 18th she developed what was thought to be pneumonia, and was transferred to the Presbyterian Hospital. The temperature was 103.2. The white blood count was 40,750, polymorphonuclears 89 per cent. The blood urea was 1.5 gm. per liter. The physical signs of pneumonia improved but several bed sores developed. A large calculus in the gall-bladder was demonstrated by X-ray. The urine contained considerable amounts of albumin and pus, and the left kidney was found enlarged. After 2 weeks Cheyne-Stokes respiration appeared and death occurred, apparently in uremia. Twenty-four hours before death the right leg became purplish, cold and edematous.

Postmortem Examination

Autopsy No. 11,469, performed 14 hours after death.

Anatomical Diagnoses: Generalized arteriosclerosis; thrombosis of arteries of right leg; gangrene of right leg; early confluent lobular pneumonia; acute tracheobronchitis; suppurative pyelonephritis and pyonephrosis, left; syphilitic aortitis; chronic cholecystitis and cholelithiasis; melanosis of colon; old salpingo-oophorectomy, left; pelvic adhesions.

Permission to examine the brain could not be obtained.

Kidneys: The left formed a sac-like mass 15 by 10 by 8 cm., weighing 110 gm. The capsule stripped easily and the surface was finely granular. The pelvis was distended with semipurulent fluid and there were several large abscesses in the substance of the kidney. The right kidney weighed 200 gm., was soft, flabby and the cortical markings were blurred, but there were no abscesses.

Microscopic Examination of Kidneys

Microscopically the left kidney shows a diffuse pyelonephritis, with the presence of numerous, short Gram-negative bacilli. The right kidney is almost free from acute suppurative changes. Autolysis is fairly marked but there appears to be little degeneration and no necrosis of the epithelial cells. Many of them, however, are

exfoliated and the lumens of the tubules are often filled with granular coagulum. There are no significant glomerular lesions and the arteriosclerotic lesions of the larger renal branches are not extreme.

The interesting feature of the kidney sections is the presence of numerous spherical globules, identical in location, size and appearance with those described in the previous case. They are found within the nuclei, but more abundantly in the cytoplasm of the epithelium of the convoluted tubules, or free in the lumen (Figs. 5 and 6).

Staining Reactions and Microchemical Tests of Refractile Bodies

1. With hematoxylin-eosin, after formalin or Zenker fixation, the refractile bodies are unstained but have a slight brownish tinge. They are, as has been stated, very refractile and sharply contoured.

2. With eosin-methylene blue the majority of the bodies, both intra- and extranuclear, stain intensely with the methylene blue. A few of the larger ones, however, retain their brownish tinge.

3. With Pappenheim's methyl green-pyronin they retain their brownish color, in contrast to the red-staining nucleoli, from which they can readily be distinguished.

4. They are unstained with scharlach R in frozen sections of formalin-fixed material. There are only occasional small fat droplets within the degenerating epithelial cells. The globules are not doubly refractive.

5. With Nile blue sulphate a faint greenish blue staining of the globules is obtained.

6. With 1 per cent osmic acid on frozen sections of formalin-fixed material, after washing in several changes of distilled water, a slight darkening of the globules is noted. They are not blackened, however.

7. With Spielmeyer's myelin stain intense blackening of both the intranuclear and cytoplasmic bodies is produced. The bodies are brilliantly and selectively brought out by this method (Fig. 7).

8. Ciaccio's method shows the presence of amorphous sudanophile masses in the cytoplasm of some of the epithelial cells. The globules are unstained.

9. After 10 days digestion of small slices of formalinized tissue with Merck's pancreatin the globules are unaffected, save that they show a tendency to stain with hematoxylin (Fig. 7).

10. The iron reaction with potassium ferrocyanide and hydrochloric acid is negative.

11. They are not dissolved by strong ammonia or by 20 per cent nitric acid.

12. Von Kossa's stain for calcium is negative.

The following microchemical tests for bismuth were applied.

1. With hydrosulphuric acid the bodies are slightly darker than in the unstained control sections.

2. Ammonium sulphide also produces darkening, but no diffuse black coloration.

3. With stannous chloride-sodium hydroxide the inclusions stain brownish black. Under the oil immersion minute black granules in active brownian motion are seen floating in a colorless menstruum.

4. With potassium iodide-sulphuric acid the bodies take a slightly more yellowish tinge than the unstained control.

5. Frozen sections treated according to the method of Komaya [1] with quinine sulphate and potassium iodide fail to give the characteristic bismuth reaction, although a deep brick red color was obtained with bismocymol on filter paper.

In addition to the refractive globules above described there is in Case 1 a considerable amount of yellowish brown pigment within the epithelial cells at the junction of pyramid and cortex. This is in the form of irregular, varying sized clumps. It does not blacken with hydrosulphuric acid or ammonium sulphide, nor does it give an iron reaction with potassium ferrocyanide and hydrochloric acid. It fails also to react with Komaya's reagent and the other microchemical tests for bismuth. It is not removed by the lipoid solvents used in dehydration and clearing, nor does it give a fat stain with scharlach R.

Chemical Determination of Bismuth in Kidneys

In view of the fact that the patient received intramuscular injections of bismuth it seemed of interest to determine the amount of bismuth still present in the renal tissues in each case.

A colorimetric determination was carried out on duplicate samples of formalin-fixed tissue, according to the method of Leonard.[2] The analysis of Case 1 gave the following results.

	Sample A	Sample B
Wet weight	8.2058 gm.	4.3730 gm.
Dry weight	1.9527 gm.	1.0368 gm.
Bismuth found	1.36 mg.	0.0727 mg.
Bismuth per 100 gm. wet tissue	16.57 mg.	16.62 mg.
Bismuth per 100 gm. dry tissue	69.7 mg.	69.8 mg.

Taking the combined weight of the two kidneys as 280 gm., the calculated total amount of bismuth retained by the renal tissue may be estimated as approximately 46.5 mg., or well over 10 per cent of the total amount injected. To what extent the extreme carcinomatous replacement of the liver tissue interfered with the normal storage of the bismuth in the reticuloendothelial cells (Komaya) of this organ, thus intensifying the toxic effect upon the kidney, must remain problematical. The finding of a considerable amount of bismuth in the kidney made it logical to ascribe the renal damage to this substance. Only a trace of mercury could be recovered.

An analysis of the kidneys of Case 2, carried out with the same technique, gave the following results.

Wet weight	4.6955 gm.
Dry weight	1.1016 gm.
Bismuth found	1.120 mg.
Bismuth per 100 gm. wet tissue	23.8 mg.
Bismuth per 100 gm. dry tissue	101.67 mg.
Total weight of both kidneys	310.0 gm.
Total bismuth content	73.8 mg.
Total amount injected	1.0 mg.

The kidney at the time of death therefore contained 7.38 per cent of the amount injected.

The nature of the refractile bodies within the epithelial cells still remained a problem. It seemed of interest to determine whether they could be reproduced experimentally or not.

Experimental Production of Similar Globules in Rats

A white rat, weighing 220 gm., was injected intramuscularly with four successive doses of bismocymol as follows.

On February 8, 1933, 0.1 cc. (equivalent to 261 mg. pro kg.) was injected, on February 9th 0.2 cc. was injected, on the 10th 0.45 cc., and on the 13th 0.4 cc. The animal showed no symptoms save a loss of weight of 30 gm. It was killed 3 days after the last injection. Bismuth was still present at the site of injection. About the material

was a grayish membrane, outside of which the muscle was edematous and hemorrhagic. The kidneys were dark red, the cortex showing grayish streaking. Other viscera were not abnormal.

Microscopically the kidney is the seat of an intense tubular necrosis. In many of the convoluted tubules the epithelial cells have lost their nuclei, stain intensely with eosin, are partially exfoliated and completely plug the tubules. There are no regenerative changes. Spherical inclusions, identical with those in the human tissue, are present both within the nuclei and in the cytoplasm (Figs. 8 and 9). They give similar microchemical reactions.

A section through the site of injection shows the bismocymol still present in the form of large granular deposits or agglomerations of spherical refractive masses having a greater variability in size than the inclusion bodies within the kidney. Treated with ammonium sulphite solution much of the deposit is immediately blackened. Many of the smaller globules take only a light brownish tint and thus closely resemble those found in the kidney. They are, however, entirely extracellular. With Komaya's reagent the injected material takes a brick red or orange color, rapidly decolorized by alkali but resistant to weak acid treatment. Some of the smaller globules react very feebly to the reagent. This suggests that the bismuth had been split off, leaving a non-reacting residue.

The injected material is surrounded by a broad zone of edematous granulation tissue. Many of the smaller globules and amorphous masses have been taken in by phagocytic cells, chiefly macrophages, but also polymorphonuclears. No globules, however, are found within the nuclei of the phagocytic cells or fibroblasts.

Chemical analysis of the rat kidney showed 0.042 mg. of bismuth in 0.1962 gm. of wet tissue, or 21.4 mg. of bismuth per 100 gm. of dry tissue.

The experiment was repeated on 7 other rats, as shown in Table I.

The inclusions were found in 5 out of 7 rats, that is, in all those surviving more than 4 days. There was no striking correspondence between the intensity of the tubular degeneration and the number of globules. Rats 11 and 15 showed only occasional tubules with necrotic epithelial cells, but intranuclear globules were numerous. The 2 rats with normal kidneys, killed on the 4th day, failed to show the globules.

The particular bismuth preparation used for treatment of the second case was not available for experimentation. Three rats were, however, given intramuscular injections of a similar preparation of sodium potassium bismuth tartrate with butyn suspended in peanut oil in doses equivalent to 153, 182 and 290 mg. per kg. respectively. None of the rats showed evidence of severe injury to the tubular

TABLE I

Summary of Injections

Rat No.	Total amount injected	Bismuth	Number injections	Days	Tubular degeneration	Globules
	cc.	*Mg/K*				
2	0.2	77.0	1	4	0	0
16	0.7	145.0	2	11	+++	+++
13	0.6	176.0	3	4	0	0
11	0.7	200.0	3	8	+	+++
1	1.15	287.5	4	8	+++	+++
15	0.82	297.0	2	5	0	++
12	1.78	393.0	4	15	+	+

epithelium, and in only 1 were there found characteristic inclusions within the nuclei. Further experiments extending over longer periods are planned. The preparation was either less toxic for the kidneys than the bismocymol or less bismuth was absorbed from the site of injection.

It seemed of interest to determine also whether similar inclusions appeared in the renal epithelial cells in response to injections of a water-soluble bismuth compound. A rat weighing 132 gm. was injected intramuscularly with 0.5 cc. of Loesser's solution of bismuth tartrate, equivalent to a dose of 107 mg. pro kg. The rat died on the 2nd day, showing extensive necrosis of many convoluted tubules but no refractile globules of the type described. Another rat, which received an injection of the same solution (0.178 mg. pro kg.) plus 0.5 cc. of a 10 per cent solution of camphorated oil into the muscles of the opposite leg, also showed severe tubular nephritis but no refractile inclusions.

Dr. G. H. Raizes of the Dermatological Research Laboratories of Philadelphia very kindly put at our disposal a sample of camphocarbonic acid, uncombined with bismuth. This was dissolved in olive oil by the aid of heat and injected intramuscularly into several

rats in amounts up to 150 mg. given in divided doses. No toxic effect upon the kidney was produced and no refractile globules were found within the epithelial cells.

Discussion

Following the intramuscular injection of 0.4 gm. of bismuth in the form of bismuth camphocarbonic acid in olive oil (bismocymol) into a syphilitic patient there occurred a tubular nephritis of moderate intensity, with regenerative change in progress at the time of the patient's death from a metastasizing gastric carcinoma. The unusual and, so far as we are aware, previously unobserved feature, was the presence of refractile spherical bodies within the nuclei and cytoplasm of the renal epithelial cells of the convoluted tubules. A second case, in which the patient had been given 1 gm. of potassium bismuth tartrate suspended in peanut oil, was found to have similar refractile globules in the epithelium of the renal tubules. The histochemical reactions in the two cases were identical.

One can say little that is definite as to the chemical nature of these bodies. Their failure to stain with sudan III or with Nile blue sulphate or osmic acid, and their resistance to lipoid solvents shows that they are not simple fats. The negative reaction to the Ciaccio method, and the fact that they are not anisotropic would indicate that they are not composed principally of lecithin-like substance or of cholesterol esters. On the other hand, the positive staining with Spielmeyer's method may suggest a chemical relation to the myelins. That they are not of a protein nature is shown by their prolonged resistance to tryptic digestion and their resistance to strong acid or alkali. The darkening with hydrosulphuric acid and ammonium sulphite suggests that they may contain traces of bismuth, but the more specific histochemical tests with Komaya's reagent and stannous chloride are negative, although the injected material at the site of injection gives clean-cut microchemical tests for bismuth.

Since similar intracellular bodies are not formed at the site of injection and cannot be detected within the glomeruli, one may assume that the material of which they are composed is eliminated from the glomerulus either in solution or in finely dispersed form, reabsorbed by the tubules, and segregated by the nucleus in the form of one or rarely two refractile globules. That the bodies are primarily formed

within the nucleus rather than the cytoplasm is indicated by the appearances in Rat 11 which received in divided doses 0.7 cc. of bismocymol, equivalent to 200 mg. pro kg. Killed on the 8th day after the first injection there was found only a slight tubular injury. Numerous globules were present in the epithelial cells, but almost exclusively within the nuclei. Often their presence was unaccompanied by any other evidence of cell injury.

In searching the literature for similar observations there was found a short paper by Kollert, Strasser and Rosner.[3] Following the administration of trepol (potassium sodium tartrobismuthate) there appeared in the urinary sediment small, polygonal, finely granular epithelial cells. The nucleus of these was usually not distinguishable, but a sharply contoured refractile body about the size of the nucleolus was often distinctly seen. No doubly refractile bodies were found with the polarizing microscope.

In the description of the tubular nephrosis produced in rabbits by toxic doses no further mention is made of these refractile bodies. Whether they were of the same nature as those described above or not cannot be decided. We have found no other reference to such structures in the rather extensive literature concerning the effects of bismuth upon the tissues.

Summary

Refractile globules were found within nuclei and cytoplasm of renal epithelial cells in 2 cases following intramuscular injection, in 1 instance of bismocymol (a bismuth derivative of camphocarbonic acid), and in the other of potassium bismuth tartrate with butyn. Similar globules were found in the renal epithelial cells of rats after the injection of appropriate doses of bismocymol. The chemical nature of these globules was not determined; they gave equivocal reactions for bismuth, were insoluble in lipoid solvents and in strong alkalis and acids, resisted tryptic digestion, did not react for iron or calcium but stained as myelin by the Spielmeyer method.

Note. We are indebted to Doctors Allen Whipple and Walter Palmer for permission to include the clinical records of these cases.

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DESCRIPTION OF PLATES

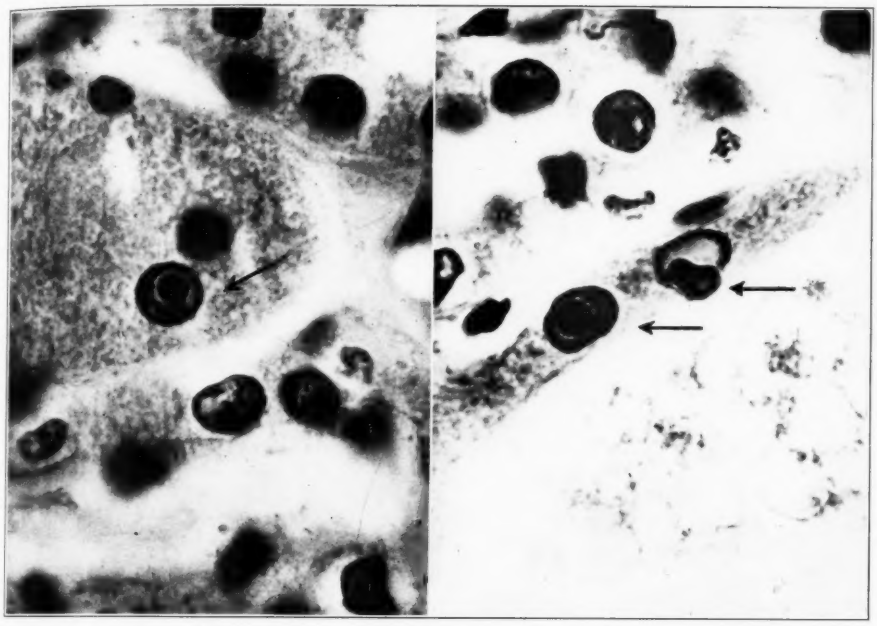
PLATE 138

FIG. 1. Case 1. Intranuclear body in renal epithelial cells. Hematoxylin-eosin stain. × 1680.

FIG. 2. Case 1. Two inclusion bodies are seen; one spherical in shape, completely within nucleus; another, pear-shaped, apparently escaping through a gap in the nuclear membrane. × 1680.

FIG. 3. Case 1. Refractile spherical inclusion in cytoplasm. The nucleus is pyknotic and irregular in shape. × 1680.

FIG. 4. Case 1. Two cytoplasmic inclusions are shown, one adjacent to a nucleus; another lies in a necrotic cell that has lost its nucleus and is desquamated into the lumen. × 1680.



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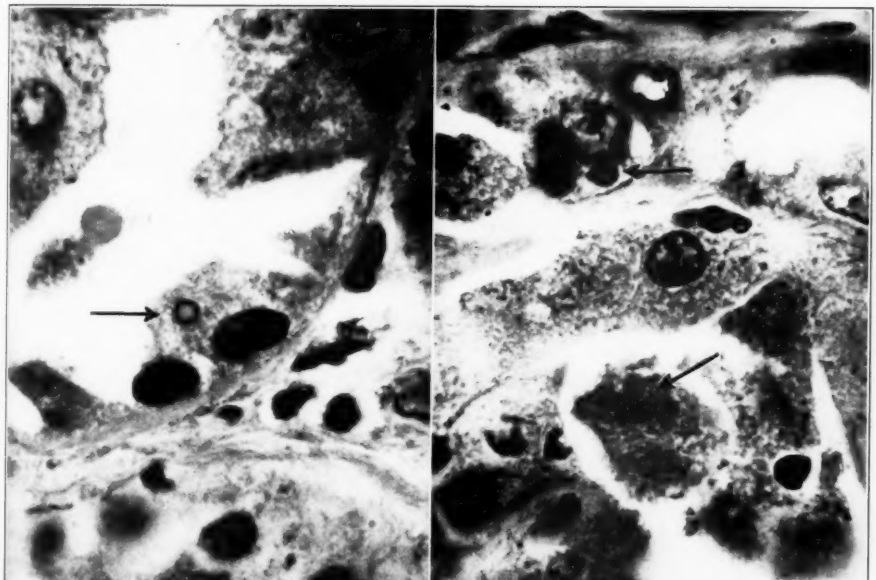


3 4

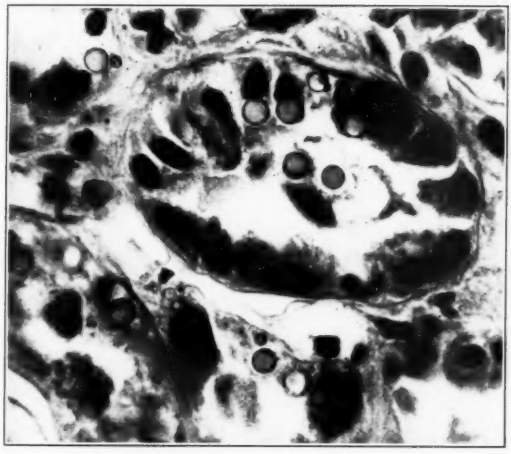
Pappenheimer and Maechling Inclusions in Renal Epithelial Cells

Plate 139

Fig. 5. Case 2. Renal tubules containing numerous inclusions, chiefly cytoplasmic. Zenker fixation. Hematoxylin-eosin stain. × 720.

Fig. 6. Case 2. Frozen section of kidney, myelin stain, Spielmeyer's method. The globules are stained black. × 100.

Fig. 7. Case 2. Formalin-fixed tissue, washed, digested for 12 days with Merck's trypsin. The outlines of the tubules are still recognizable. The globules resist digestion and stain with hematoxylin. × 700.



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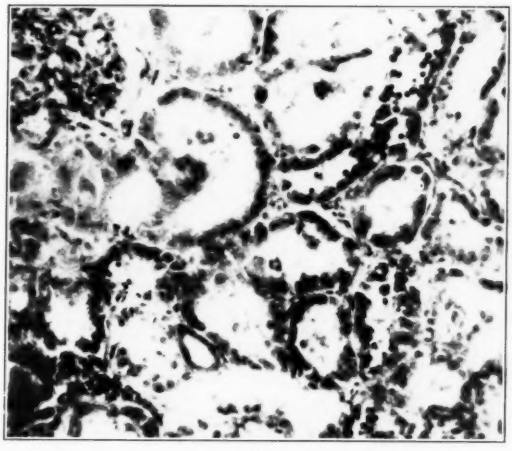

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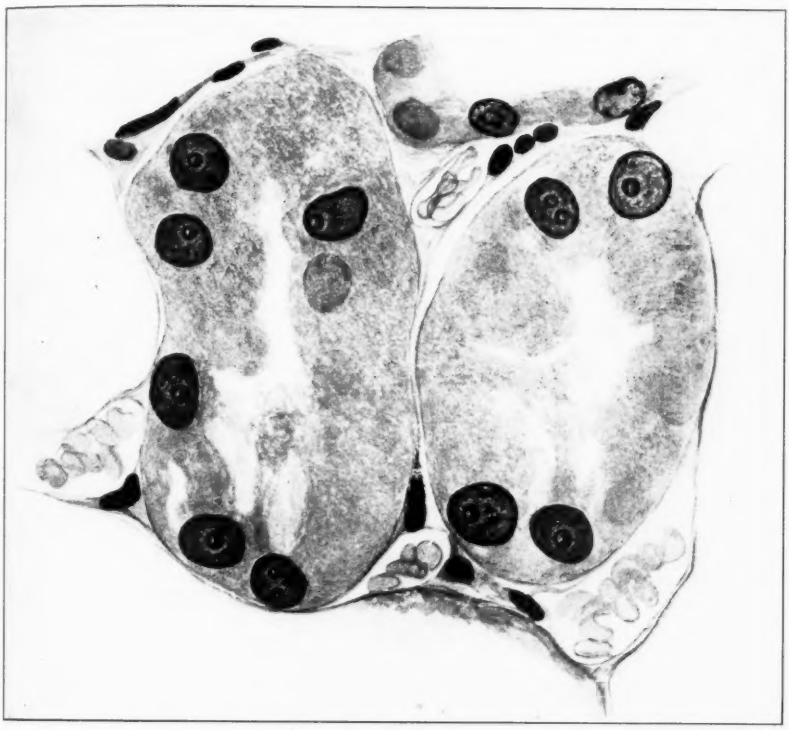
PLATE 140

FIG. 8. Rat 15. Numerous refractile globular inclusions within nuclei of renal epithelial cells, following injection of bismocymol. Zenker fixation, hematoxylin-eosin stain.

FIG. 9. Rat 15. Kidney with intranuclear inclusions. Unstained frozen section after formalin fixation. × 720.



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GLYCOGEN-STORAGE DISEASE *

THESAURISMOSIS GLYCOGENICA (VON GIERKE)

ELEANOR M. HUMPHREYS, M.D., AND KATSUJI KATO, M.D.

(From the Departments of Pathology and Pediatrics, University of Chicago, Chicago, Ill.)

Glycogen-storage disease was first described by von Gierke [1] in 1929 when he reported the clinical and autopsy observations on 2 cases. Their most unusual feature was the large size of the liver and kidneys, and the direct cause of enlargement was the accumulation of glycogen in quantities far beyond those commonly observed. Glycogen was found principally in parenchyma cells, a point of difference from most storage disorders where the reticuloendothelial system is the principal depot. In a subsequent report [2] von Gierke emphasized the storage aspect of the condition and proposed the descriptive name "*Thesaurismosis glycogenica.*" His report and the records of 10 proved cases reported since 1929 provide a basis for outlining the main features of this interesting disease. A disorder of infancy and childhood, it seems to be the result of defective metabolism, or at least defective mobilization of carbohydrates. The exact mechanisms concerned are obscure, but we know that glycogenic infiltration is not confined to the organs that are obviously enlarged. The type first described by von Gierke, in which the liver and occasionally the kidneys are hypertrophied and infiltrated with glycogen, has been designated as hepatomegalic or hepatonephromegalic. Bischoff [3] and Putschar [4] first described a cardiomegalic type. We wish to present observations on 4 cases, 3 of them proved and 1 a probable example of cardiomegalic glycogen-storage disease.

CASE REPORTS

CASE 1. *Clinical History:* E. L., a male infant, and the first child of young, healthy parents, weighed 9¾ pounds when born on Oct. 20, 1932. Seemingly normal at birth, he took feedings poorly and often was constipated. When 3 months old he had "influenza," after which he coughed for 1 month. He gained weight slowly and weighed 14 pounds at 4½ months. He was brought to the

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Bobs Roberts Memorial Hospital on March 15, 1933, because of moderate fever and cough of 4 days duration.

The infant appeared malnourished, dehydrated, listless and extremely weak. His temperature was 37.8° C. The skin was dry and cold and the tissue turgor poor. Cyanosis was observed, breathing was labored, and expansion of the chest was limited on the left side. Râles were heard over both lungs and there were signs of consolidation of the left lung. The heart was enlarged and a soft, blowing systolic murmur was heard at the apex and in the left midaxillary line. A roentgenogram of the chest confirmed the impression that the heart was greatly enlarged and the left lung consolidated. The liver and the tip of the spleen were palpable. The scrotum was large and cystic and the head of the right epididymis was firm. The erythrocyte count was 3,300,000, with 75 per cent hemoglobin. The leukocytes numbered 15,800 with 51 per cent neutrophiles, 39 per cent lymphocytes, 8 per cent monocytes and 2 per cent eosinophiles. Examination of the urine was negative except for the presence of a few leukocytes. Wassermann and Kahn tests on the blood of the parents and a Kahn test on that of the infant were negative. The temperature rose to 39.4° C before death, which occurred on March 16, 1933.

The clinical diagnosis was left lobar pneumonia and probable right bronchopneumonia; cardiac hypertrophy with mitral regurgitation, based on an organic (congenital) defect; and bilateral hydrocele.

Autopsy Report

The autopsy was performed 12 hours after death. The body was 64.8 cm. long, and weighed 6.14 kg. The lips and nailbeds were cyanotic. The skin was pale and loose, and edematous only in the scrotum. The chest was symmetrical save for a bulging right costal border below which the liver was felt. The abdomen was distended and the extremities appeared thin. Subcutaneous fat was scanty and the muscles of the trunk were extremely pale. The margin of the liver was 3 cm. below the right costal border. When the sternum was removed the heart was found to be enormously enlarged, with its left border in contact with the lateral chest wall and its right border 1 cm. to the right of the sternum. The left lung was displaced backward and upward. The thymus, a fleshy organ weighing 28 gm. (normal range 6.3 to 19.3 gm.[5]) covered the upper half of the pericardium. The pericardial sac and the left pleural cavity each contained 10 cc. of clear fluid.

The heart weighed 140 gm.* (29 gm.[6]). Measured *in situ* the length from the base of the ventricles to the apex was 10 cm., while

* In this and subsequent notations the "normal" weights (cited in parentheses after those observed) are the mean weights of organs of children in the corresponding age group, as given in the tables of Coppoletta and Wolbach.[6] Where the age fell between two listed groups the weights for the older group were chosen.

the greatest transverse and anteroposterior diameters were 7.5 and 5 cm. The apex was broad and the left margin and the anterior wall were rounded. The atria and auricles were small, but the walls of the ventricles were firm and thick and their large muscle structures encroached on the cavities. Measured between the muscle columns the right ventricle was 4 to 6 mm. and the left 1 to 1.4 cm. thick. The intact interventricular septum was 1 to 1.2 cm. thick. A probe could be passed through a small channel at the margin of the foramen ovale but the ductus arteriosus was closed. The valve circumferences were: tricuspid 6.2 cm., pulmonic 4 cm., mitral 5 cm., and aortic 3.5 cm. Marginal fenestrations were present in both sets of semilunar cusps. Both the mural endocardium and the epicardium were opaque. The exceptionally pale myocardium was reddish pink mottled with pinkish gray. A wide pale zone beneath the endocardium of the left ventricle had a peculiar glassy appearance. The coronary arteries were large, but like the large arteries and veins they were normally formed.

The lungs were hyperemic and wet and the posterior third of the left lung was atelectatic. Beneath a group of pleural petechiae in the left lower lobe were several small, red, consolidated patches. The liver weighed 260 gm. (188 gm.) and had a smooth capsule. Its tissue was pale yellow-pink, with indistinct lobules, and was soft but not friable or greasy. The two kidneys weighed 60 gm. (50 gm.) and were cyanotic. The spleen, weighing 20 gm. (16 gm.), had firm, red-purple pulp and large malpighian bodies. The lymphoid tissue of the lower ileum and colon was hyperplastic, and the mesenteric lymph nodes were moderately enlarged while the other visceral nodes were small. Together the adrenal glands weighed 3.4 gm. and each had a thin pale cortex and a red-brown medulla. The tunics of both testicles were distended by clear fluid and a cystic hydatid was attached near the head of the right epididymis. The brain was not examined.

Microscopic Examination

Technique: Fourteen hours after death tissues were placed in Zenker's solution and in formalin. Scarlet R was used to demonstrate fat. The complete series of Zenker-fixed tissues was embedded in celloidin and stained with hematoxylin and eosin. Sections of striated muscles were also stained with Mallory's phosphotungstic

acid hematoxylin. Lacking material properly preserved for demonstrating glycogen the tissues fixed in formalin (heart, liver, kidney) were embedded in celloidin and stained with Best's carmine. Subsequently this stain was applied to the Zenker-fixed tissues. Where sections of the same organ were compared glycogen was more abundant in the tissues fixed in formalin. The nature of the granules stained by carmine was checked by the simultaneous staining of control sections. These included tissues known to contain glycogen, glycogen-free tissues, and in some instances saliva-digested sections of the tissues under investigation.

Because of poor fixation and the interval of 1 week between fixing and embedding the tissues, positive observations were more significant than negative ones. Where glycogen was abundant large aggregates of coarse and fine red granules completely filled vacuolated cells, but in other regions their locations suggested diffusion artefacts. We do not know whether or not artefacts are to be blamed for the occasional presence of granules in the interstitial tissues and in the lumens of ducts and blood vessels. Proof that some glycogen had escaped into the formalin preserving fluid was afforded by its milky opalescence. Treated with alcohol this fluid yielded a white precipitate with the physical and chemical properties of glycogen.

Heart: The outstanding features are hypertrophy and vacuolization of the muscle fibers. The largest, up to 50 microns in diameter, lie close to the endocardium, and some of these probably are fibers of the Purkinje type. In the large muscle columns of the left ventricle many fibers measure 30 microns, while in the outer part of the wall most of them are between 20 and 25 microns in diameter. Most of the diameters in the wall of the right ventricle are between 15 and 20 microns, and in the left atrium between 10 and 15 microns. In comparison few fibers measuring more than 12 microns are found in a number of hearts of infants, 5 to 12 months old.

In cross-sections almost all the fibers appear as round or polyhedral spaces surrounded by thin mantles of dots, the cut ends of myofibrils. Where nuclei are transected they are sometimes located in the center of the axial cytoplasm, more often displaced peripherally. They appear normal but some are unusually large. Longitudinal striations are distinct in fibers cut obliquely or lengthwise, but the fibrils often appear teased apart and broken off, especially where they approach large vacuoles. While the appearance of cross-

sections indicates that the axial spaces are continuous, in longitudinal sections they vary in width so that the fibers bulge irregularly. The apparent fragmentation of the fibrils is probably due to changes of direction caused by this bulging. Cross-striations are well defined in some fibers, indistinct where the fibril mantles are thin. Intercalated discs are not seen.

Glycogen granules completely fill many of the fibers, and in others form crescents or rings about clear spaces. Granules are coarser and less uniform in size and distribution than in control sections. They fill every cell in the zone that grossly had appeared glassy. A few fine granules are seen in the interstitial tissue and in the lumens of blood vessels. The muscle fibers do not contain fat or lipoids. The endocardium is moderately thickened and fibrous.

Skeletal Muscles: Comparable changes are found in the skeletal muscles where they are responsible for an unusual type of vacuolar degeneration, which we believe has not been described previously. In some fibers the vacuoles are so large that cross-sections are at first mistaken for fat cells. Similar changes, varying only in degree, are found in the cremasteric, rectus abdominis and intercostal muscles and in fragments of the pelvic muscles. Measurements of fibers of the rectus abdominis are as follows: average diameters for relatively normal fibers 25 microns, for swollen hyalinized fibers 35 microns, range for moderately vacuolated fibers 30 to 45 microns, for extremely vacuolated fibers 50 to 90 microns, with most of them between 50 and 70 microns. "Normal" measurements for comparison are not available. The muscle fibers of the newborn infant are said to measure 6 to 15 microns; the diameters of adult muscles show a wide range, from 17 to 100 microns, with the majority between 40 and 70 microns.

In cross-sections few of the fiber sheaths are filled in orderly fashion by the cut ends of myofibrils. In a few small fibers there are single centrally placed vacuoles, but in the majority the cut ends appear as reticular networks with round or irregular spaces separating fibril bundles. Fibrils are sometimes in contact with the sarcolemma and sometimes separated from it by an empty slit or ring. The peripherally located nuclei of the less vacuolated fibers are normal, but in those with large vacuoles the nuclei often are displaced from the sarcolemma, and often are pyknotic and distorted. Interspersed with fibers of these types are empty or almost empty rings, thought

to be fat cells until longitudinally cut sections failed to demonstrate fat infiltration. The discovery of an intermediate type of fiber identifies these rings as muscle sheaths. In this type the rings contain pyknotic and shrunken nuclei surrounded by halos of granular and fibrillar débris. Some of these tangled fibrils are beaded, almost certainly identifiable as degenerating myofibrils.

In fibers cut lengthwise the cross-striations are distinct when the sheaths are well filled with myofibrils. In many swollen sheaths the fibrils are grouped in thin and irregularly coursing bundles and cross-striations often are hazy or represented by unevenly spaced dots. Obviously the completely empty sheaths cannot be recognized with certainty but the intermediate type is easily identified. In the latter, pyknotic nuclear remnants with their halos of débris are distributed throughout the almost empty, irregularly bulging sheaths. In some, the granular débris is arranged so that it appears to surround vacuoles or unstained granules. In others, a few remnants of myofibrils still show cross-striations.

Coarse and fine glycogen granules are demonstrated in many of these vacuoles and clumps of granules almost fill some of the nearly empty sheaths. In the sections of cremasteric muscle nearly every vacuolated fiber is well filled with glycogen. In other muscles granule-filled fibers are found side by side with seemingly similar fibers in which no glycogen is found.

Liver: All the hepatic cells have pale foamy cytoplasm, and their small vacuoles and cell membranes are outlined by eosin. Most of the vacuoles are smaller than the nuclei, and in a few cells the nucleus is displaced peripherally. The sinusoids are narrow, and the nuclei stain normally. Fine glycogen granules fill many of the cells and in others are concentrated in crescentic masses along one side. Fine granules are present in the epithelium of the bile ducts and in the smooth muscle cells of the blood vessels. A few are seen in the interstitial tissues and in the vascular lumens. Fat is demonstrated in only a few liver cells.

Kidneys: Precipitated protein is present in the glomerular spaces and tubules. The cells of the medullary tubules are unusually pale and swollen and contain considerable glycogen. In smaller quantities glycogen is present in the epithelium of the loops of Henle and of some of the convoluted tubules. As in the liver, vascular smooth muscle cells contain glycogen, and coarse red granules and clumps

lie in the lumens of a few medullary tubules. A few of the large pale epithelial cells contain granules which are stained orange by scarlet R, while red fat droplets are abundant in the epithelium of the cortical tubules.

Lungs: In sections from the left lung we find hyperemia and edema, atelectatic foci and bronchopneumonic patches. A little glycogen is observed in the cells of the bronchial cartilages.

Lymphoid Tissues: In the mesenteric lymph nodes, the thymus, the ileum and the spleen the lymphoid tissues are hyperplastic. In the last two the lymph follicles have centers of large pale cells and fragmented nuclei. Glycogen is found in some of these pale cells and in large cells in the medulla of the thymus.

Smooth Muscles: Axial vacuoles are seen in smooth muscle cells of the urinary bladder, the ductus deferens and many blood vessels, but not in the muscle cells of the ileum. Many of these vacuoles are 10 to 12 microns in diameter and occupy most of the cross-section of the fiber, so that the affected muscle is pale and lacy. No glycogen is found, excepting in the vascular smooth muscle of the liver and kidneys.

Other Organs: Glycogen is not found in the other tissues examined. The thyroid gland has small, colloid-filled acini lined by low cuboidal epithelium. A few small hemorrhages are seen in the medullas of the adrenal glands. The pancreatic islets are not unusual but the acinar cells stain very irregularly because some are well filled with zymogen granules, while others have a pale vacuolated cytoplasm. The nature of these vacuoles, which are often as large as the nuclei, cannot be determined. The genital organs are normal for the age.

CASE 2.* *Clinical History:* G. H., a male twin 8 months old, was brought to the Rockford Hospital on March 27, 1933, because of an acute illness with fever. He had developed normally for the first 2 months, and then had a series of respiratory infections, diagnosed as pneumonia on three occasions. He had been restless, had a poor appetite, and not only had failed to gain, but recently had lost weight. He weighed 12 pounds as compared with the weight of his "control" twin, 19 pounds.

The child was acutely ill and had a fever of 100° to 104° F. The liver was enlarged, and both physical examination and a roentgenogram demonstrated un-

* Our attention was directed to this case by Dr. Joseph Brennemann of Chicago, and we are indebted to the attending physician, Dr. W. L. Crawford, and the pathologist, Dr. H. D. Palmer, both of Rockford, Ill., for the data presented. They also permitted us to examine the heart and prepare sections of the myocardium.

usual cardiac enlargement. Bronchial breathing was heard on the left side posteriorly, but the lung fields visible in the X-ray film were clear. The urine contained no acetone or diacetic acid. Two blood cultures remained sterile. On March 29th the erythrocyte count was 4,400,000 with 79 per cent hemoglobin, and on March 31st 3,650,000 with 78 per cent hemoglobin. The corresponding leukocyte counts were 16,850 and 15,050, with 41 and 51 per cent neutrophiles. Cyanosis appeared before death, which occurred on March 31st.

The clinical diagnosis was unexplained cardiac hypertrophy, possibly with endocarditis.

Autopsy Report

The body looked like that of an infant 4 or 5 months old. Edema was absent and the skin was wrinkled because of the scarcity of subcutaneous fat. The abdomen was distended. Each pleural cavity contained 25 cc. of fluid. *In situ* the greatest width of the heart was 9 cm., as compared with the maximum transverse diameter of the chest, 14 cm. The heart was globular and had thick-walled ventricles, small cavities and normal septa and valves. At its thickest part the right ventricle measured 8 mm., the left 3.2 cm. (after fixation 1.7 to 2.2 cm. in the regions between its huge muscle columns). The heart and lungs together weighed 360 gm. and the net weight of the heart was estimated as 260 gm. (37 gm.). The thymus weighed 5 gm. The left lung was compressed and the right contained pneumonic patches. The liver was large, contained much blood, and extended four finger-breadths below the ribs. The kidneys were of normal size.

Microscopic Examination

The findings are very similar to those in Case 1. In the lungs are atelectatic foci, edema, hyperemia and small pneumonic patches. The liver cells are filled with small vacuoles but contain no fat. The tubular epithelium of the kidney is swollen and minutely vacuolated. The general architecture of the myocardium is unchanged, but there is a striking hypertrophy with vacuolization of the muscle fibers without fatty change. Sections were prepared from various parts of the heart, after it had been kept in preserving fluid for 5 months. We find the myocardial fibers differing from those of Case 1 only in that they and their more vesicular nuclei are larger. With the carmine stain their vacuoles are well filled with glycogen. The preserving fluid also contains considerable glycogen.

CASE 3.* *Clinical History:* L. W., a female infant, 4 months old, weighed 7 pounds and seemed normal at birth. For 1 month before admission to the Babies Hospital on Dec. 10, 1922, she had a cough and lost weight. For 1 week her ankles and wrists had been swollen. Fever was absent. She was admitted in a moribund state with physical signs suggesting pulmonary consolidation, and died 9 hours later.

Autopsy Report

The nutritional state was poor, edema was present in the extremities, and there were many small dermal angiomas. The heart, large and globular, weighed 90 gm. (27 gm.). The left ventricle was 1.5 to 2 cm. thick, the right 5 to 9 mm. The papillary muscles were large and the ventricles were not dilated. The myocardium of the right ventricle was firmer than that of the left and was pale pink. The lungs contained regions of congestion and of atelectasis, and were emphysematous anteriorly. The liver and kidneys were congested and were not enlarged. The liver was flabby and seemed somewhat fatty. The spleen was firm and contained conspicuous malpighian bodies. The lymphoid tissues were not hyperplastic. A few petechiae were seen in serous membranes.

Microscopic Examination

Sections of the liver and heart are almost identical with those of the preceding cases. There is little fat in the liver. The muscle fibers of the diaphragm are vacuolated, resembling the moderately vacuolated skeletal muscle of Case 1. There is swelling of the epithelium of some of the renal tubules. The spleen has large malpighian bodies with centers of large pale cells and there are similar cells in the pulp. According to the protocol glycogen was demonstrated in formalin-fixed sections of the heart, but not in those of the liver.

CASE 4.† *Clinical History:* K. B., a female infant, 5½ months old, was admitted to the Harriet Lane Home on May 7, 1919, because of cough, weakness, restlessness and loss of weight during the past 2 months. This baby was weak from birth and had pneumonia at the age of 7 weeks. She was fairly well developed but the nutritional state was poor. The left side of the chest bulged

* This case is included with the consent of Dr. Martha Wollstein of New York City, who sent us microscopic preparations and the protocols. Both this and the following case were found by one of us in a survey of hearts showing unexplained hypertrophy.

† This case, which must be classed as presumptive because the presence of glycogen was not proved, is included by permission of Dr. Edwards H. Park and Dr. Arnold Rich of Johns Hopkins University.

and showed diminished expansion and dullness. No râles were heard and the heart sounds were clear and regular. A roentgenogram confirmed the suspected cardiac enlargement and revealed clear lung fields. The spleen and the liver were palpable. Dyspnea, high fever and increasing prostration preceded death at the age of 6 months.

The clinical diagnosis was idiopathic hypertrophy of the heart.

Autopsy Report

The heart weighed 128 gm. (31 gm.). The wall of the right ventricle was 5 mm. thick, that of the left 1.7 cm. The papillary muscles were large. Valves and septa were normal and the ventricles were roomy but not dilated. There was no evidence of pneumonia. The liver weighed 290 gm. (200 gm.), the spleen 10 gm. (17 gm.) and the two adrenal glands 4 gm.

Microscopic Examination

The hypertrophy and axial vacuolization of the myocardial fibers are of the type seen in the other three hearts. Transverse striations are often indistinct and in some fibers the myofibrils appear finely granular. The muscle cell nuclei are vesicular. The hepatic cells and the cells of the collecting tubules of the kidneys are pale and foamy. The lymphoid bodies of the spleen are small but some have centers of large pale cells. The lungs are edematous but no pneumonic foci are found.

Review of the Literature

Proved Cases of Glycogen-Storage Disease

Because the criteria for clinical diagnosis are not well established, only those cases where unusual quantities of glycogen have been demonstrated in hypertrophied organs can be listed as proved cases. Von Gierke's [1] 2 cases were examples of the hepatonephromegalic type of the disorder. Chemical analyses of the tissues of his first patient were reported by Schönheimer.[7] Unshelm [8] (Case 2) and Kimmelstiel [9] described a case which might be designated as hepatomegalic, since the only significantly enlarged organ was the liver. Beumer and Loeschke,[10] Loeschke,[11] and Schall [12] added 2 cases of the same type, where the diagnoses were based on hepatic biopsies. Bischoff [3] and Putschar [4] reported the first example of the cardiomegalic type. Pompe [13] described a similar case, studied chemically

by van Creveld.[14] Pompe also gave brief notes on 3 other cases similar to his own. Antopol, Heilbrunn and Tuchman [15] have recently described 1 case, and the case reported by Sprague, Bland and White [16] should almost certainly be included in this group*. With the 3 cases here reported there are 5 proved examples of the hepatic-renal or hepatic type and 10 of the cardiac type. The essential data are presented in the following summary and table.

Summary: Aside from some of the exceptionally large percentages of glycogen the most unusual feature has been the extent of enlargement of the most affected organs. Livers weighing three to four times and hearts three to seven times the normal weights have been described. These figures are conservative, since the "normal" weights represent mean values for organs of children in the same age groups, while most of these children were subnormal in development and nutritional state. In most instances death was preceded by a severe respiratory infection. There were no significant anatomical abnormalities. Fats and lipoids were present in moderate amounts in some of the glycogen-rich organs, but often were absent. Their presence may have been partly responsible for hepatic enlargement in some cases.[1] Inflammatory lesions were not associated with glycogen-storage and usually there were no proliferative changes. However, two of the affected livers had an increase of fibrous tissue, suggesting an early cirrhosis,[1, 8, 9] and in our Case 1 the endocardium was thickened. Retrogressive changes accompanied by nuclear degeneration were definite only in the skeletal muscle of the same patient. Possibly the indistinct and granular appearance of the myofibrils in some of the hearts represented early degenerative change. The thymus and lymphoid tissues have been described as hyperplastic and as atrophic. The endocrine organs usually have been considered normal, although in 1 case the adrenal glands were thought to be small [1] and other observers concluded that there were slight abnormalities in the islets of the pancreas. In most of the cases the brain was not studied in detail but in 1 instance it was especially rich in glycogen.[8, 9]

Aside from the greatly enlarged organs there were differences in the distribution of glycogen. It was found in the heart in only 1 case

* A personal communication from Dr. Tracy B. Mallory, who performed the autopsy, states that carmine-stained granules were demonstrated in nearly every vacuolated fiber of the formalin-fixed myocardium.

TABLE I

Proved Cases of Glycogen-Storage Disease

Case	Age	Sex	Liver		Kidneys		Heart		Glycogen in other tissues
			Weight or size	Glycogen	Weight or size	Glycogen	Weight or size	Glycogen	
			gm.	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	
von Gierke [1] Case 1 Schönheimer [7]	8 yrs.	F	2000 *(736)	10.43 †(33.72)	245 (149)	6.53 †(36.82)	80 (110)	?	A little in skeletal muscle, cartilage cells, thymus
von Gierke [1] Case 2	4 yrs. 10 mos.	M	1860 (596)	4.32	Enlarged	Abundant	Slightly enlarged	?	In leukocytes
Unshelm [8] Case 2 Kimmelstiel [9]	20 mos.	M	1600 (370)	14.20 †(47.68)	90 (87)	0.81	50 (56)	Abundant	Abundant in skeletal muscle, brain
Beumer and Loeschke [10] Loeschke [11]	3 yrs.	M	Greatly enlarged	Abundant (biopsy)	....	....	....	....	....
Schall [12] Case 1	8½ yrs.	F	Greatly enlarged	Abundant (biopsy)	....	....	....	....	None found in skeletal muscle (biopsy)
Bischoff [3] Putschar [4]	4 mos	F	240 (160)	Abundant	50 (43)	Abundant	110 (27)	‡Abundant	....
Pompe [13] van Creveld [14]	7 mos.	F	Not enlarged	9.13	Not enlarged	Abundant	190 (34)	7.96	Skeletal muscle 9.39%; spleen 1.46%; adrenals 1.25%; spinal cord 0.58%; lung 0.34%

Pompe [13] (van Rijssel)	6 mos.	?	?	?	?	?	140 (31)	Amount not stated	....
Pompe [13] (Deelman Case 1)	4 mos.	?	?	?	?	?	110 (27)	Abundant	....
Pompe [13] (Deelman Case 2)	9 mos.	?	?	?	?	?	110 (37)	Abundant	....
Antopol, Heilbrunn and Tuchman [15]	4½ mos.	M	Enlarged	§3.25	Enlarged	§ 4.34	85 (29)	§3.57	....
Sprague, Bland and White [16]	7 mos.	F	?	|| ?	?	|| ?	175 (34)	Abundant	....
Humphreys and Kato Case 1	5 mos.	M	260 (188)	Abundant	60 (50)	Abundant	140 (29)	Abundant	Abundant in skeletal muscle; a little in cartilage cells, smooth muscle, lymphoid tissue, thymus
Case 2	8 mos.	M	Enlarged	|| ?	Not enlarged	|| ?	260 (37)	Abundant	....
Case 3	4 mos.	F	Not enlarged	|| ?	Not enlarged	?	90 (27)	Present	|| Skeletal muscle?

* Normal weights (in parentheses after observed weights) from the tables of Coppoletta and Wolbach [6] represent the mean organ-weights of children of corresponding ages.
† Percentage of weight of dried tissue.
‡ Probably about 6 per cent glycogen.
§ Analyses of organs kept in formalin for 1 month.
|| Type of vacuolization suggestive of glycogen infiltration.

of the hepatic group,[8,9] but we are unable to state whether it was sought in the other 2 fatal cases. In the kidneys of the first child, and in those of infants of the cardiomegalic group, moderate quantities of glycogen were found, mainly in the epithelium of the medullary tubules. In contrast, von Gierke found much glycogen, chiefly in cortical tubules, in the enlarged kidneys of his two patients.[1] Further evidence of variation from case to case is offered by the comments on skeletal muscles. These were reported as containing little,[1] much,[8,13] and no glycogen,[12] the last the report for an alcohol-fixed biopsy specimen. Part of the variability is probably attributable to technical imperfections. In several cases where glycogen was demonstrated only in the heart it seems likely that the pale foamy cells of the liver and kidneys also contained this substance; at least these cells were similar to hepatic and renal cells in which glycogen was abundant.

The resistance of the glycogen in these tissues to postmortem hydrolysis has attracted much attention. It has been demonstrated in large amounts despite such unfavorable factors as preceding febrile and toxic states and intervals up to 24 hours between death and autopsy. Some of the large percentages recorded in the table represent analyses of tissues kept for several days in the ice-box.[7,14] In 1 case hepatic glycogen decreased only from 14.2 to 13.67 per cent after 7 days storage.[8] Schönheimer [7] found very little glycogenolysis in a liver incubated for 1 week, while the glycogen of a control specimen decreased to one-fifth of the original. Van Creveld[14] observed a decrease of cardiac glycogen from 7.96 to 6.74 per cent after incubating for 3 days at 37° C, and to 1.7 per cent in the same interval, after mixing with normal cardiac muscle. Other observers noticed the same susceptibility to digestion by the enzymes of normal tissues, and, after extraction, by saliva.[7]

Other findings of interest were increased blood glycogen,[1,14] high blood diastase,[1] and liver amylase.[8] The possibility of familial occurrence was strongly suggested in 2 cases.[8,16]

Probable Cases of Glycogen-Storage Disease

Hepatic Type: Recently reported cases which resemble in many respects the proved cases of this type have been described by Snapper and van Creveld,[17] van Creveld,[18,19,20] Thoenes,[21] Exchaquet [22] (3 cases), Schall [12] (2 cases), Hertz,[23,24] Biedermann and Hertz[25] Zel-

son and Rauh,[26] Worster-Drought and Weber,[27] Warner,[28] Smith and O'Flynn [29] (2 cases), and Unshelm (Case 1).[8] With the exception of the last 3 these children were living when the reports were published. Their ages ranged from 20 months to 12 years. Both sexes were represented almost equally. A number of the older children were improved and seemed to be recovering after periods of observation covering several years. Evidences of hypophyseal dysfunction were thought to be present in 2 cases.[17, 23] Unshelm's [8] 2 patients were siblings, as were Smith and O'Flynn's [29] 2 patients and Exchaquet's [22] 3. Interestingly the last-named were 4 year old twins of different sexes and their 12 year old brother, who seemed to be recovering from a disturbance similar to theirs.

The only autopsy in this group was reported by Smith and O'Flynn.[29] An undernourished boy, 6 years old, had a liver weighing 2500 gm. (642 gm.) which had "plant-like" pale cells. They failed to demonstrate glycogen (method?) but found only a little fat in the vacuoles. They quoted the report of the autopsy on the patient's 20 months old sister who had a very large liver "in a state of extreme fatty degeneration." This report did not specify the use of fat stains. That the large fatty liver and the large glycogen-rich liver may be confused is evident from the original diagnosis in von Gierke's Case 2.[1] In 2 other cases [8,17] a diagnosis of enlarged fatty liver was made at exploratory operation but was not confirmed by microscopic study.

Cardiac Type: No case of this type has been diagnosed during life. Since almost every heart in the group of proved cases was originally thought to be an example of idiopathic cardiac hypertrophy we might expect to find other cases of glycogenic cardiomegaly masquerading under this diagnosis. We have little doubt that our Case 4 should be listed here, and other hearts with suggestive descriptions are the one reported by Frola [30] and possibly that of Steiner and Bogin.[31] However, a review of the histological descriptions in other case reports and the study of other enlarged, normally formed hearts have convinced us that a group of unexplained or idiopathic cardiac hypertrophies remains.

One other class of abnormal hearts, which may include examples of glycogen-storage disease, is the group of the cardiac rhabdomyomas, particularly the diffuse type. Case 3 of this report was tentatively placed in this group. Certainly Schmincke's [32] description

reads like the descriptions of the hearts we have studied. Rehder's [33] case seems to represent a transition between the diffuse and the more common nodular rhabdomyoma. Pompe [13] has suggested that even the nodular forms may represent localized glycogen-storage disease as their vacuolated fibers are similar and have been shown to contain glycogen. In our cases, however, there were none of the supposedly characteristic "spider cells" (Cesaris-Demel) and none of the appearances suggesting the evolution of myofibrils, so well described by Wolbach.[34] The absence of these features and of the extracardiac lesions commonly associated with rhabdomyomas, and the presence of vacuoles in skeletal as well as cardiac muscle, led Dr. Wollstein to doubt whether her specimen (Case 3) should be classified as diffuse rhabdomyoma.

The question of the possible survival to later years of patients with glycogenic cardiomegaly is raised by the reports of Uehlinger [35] and Levy and Rousselot.[36] Uehlinger attributed to diffuse cardiac rhabdomyomatosis the otherwise unexplained cardiac hypertrophy of a 20 year old laborer who died of tetanus. The heart weighed 340 gm. and its large, vacuolated, fat-free fibers contained much glycogen. It was thought to resemble Rehder's specimen more than Schmincke's. The other patient, an 18 year old schoolboy, died of coronary embolism. The entire myocardium of the 750 gm. heart showed a peculiar vacuolar or "hydropic" degeneration, and few vacuoles contained fat. There were fibrous scars and infarcts in this heart, without evident arteriosclerosis. Although large, vacuolated, glycogen-rich fibers often may be demonstrated at the margins of infarcts, this report emphasized the universal distribution of vacuoles. Moreover, save for scarring, the photomicrographs accompanying the report are almost identical with those of our cases.

Possibility of Other Types: Van Creveld [19] discussed the possible occurrence of types with other major localizations and of local types. He cited Deelmann's observation of glycogen-rich, hypertrophied smooth muscle cells, once in the pylorus of an infant with congenital pyloric stenosis and once in that of a woman 31 years old, who had gastric complaints from infancy. Van Creveld failed to find glycogen in a second case of infantile pyloric stenosis. This association of hypertrophy and glycogen-richness may or may not be significant. We know little of glycogen in smooth muscle, save that it may be found in normal cells.[37]

Effects of Glycogen-Storage Disease

Cardiac Type: For the most part these infants were normal at birth, but weakness and retardation of development were noticed early and difficulties in feeding were common. Respiratory infections were frequent, often recurrent. Less constantly observed abnormalities were hepatic enlargement, constipation, flabbiness of the muscles, periods of rapid breathing and of cyanosis, tachycardia, cardiac murmurs and edema. The only electrocardiogram showed a sino-auricular tachycardia and normal axis deviation.[16] No metabolic studies have been made, but in contrast to the other group, ketonuria has not been observed.

Hepatic Type: These children, too, seemed normal at birth, but after periods of several days to 1 year abdominal or hepatic enlargement was noticed. Retardation of growth was evident after a few months, with height increase lagging more than weight increase. In some cases the bones were delicate and epiphyseal differentiation was delayed. Usually the muscles seemed fairly well developed but flabby, and the amount of subcutaneous fat was variable. Often walking was delayed. Mental development was unaffected and symptoms referable to the heart and kidneys were negligible. Carbohydrate hunger was a symptom often commented on by the parents. Many of these children had recurrent infections, usually of the respiratory tract.

Metabolic studies were most complete in the 2 proved cases diagnosed by biopsy [11,12] and in certain of the presumptive cases. Some of the abnormalities of the carbohydrate metabolism are surprising in view of the large stores of glycogen present in the body. The blood sugar in the fasting state has always been low, usually less than 60 mg. per cent. Moreover, even with concentrations as low as 19.5 mg. per cent (Folin) the symptoms commonly associated with spontaneous hypoglycemia were absent. Usually a ketonuria was present in the fasting state, and ketone bodies disappeared slowly after eating. In a few instances ketosis was associated with vomiting. One of the most unusual features has been the failure of adrenalin to induce an appreciable increase of blood sugar or blood lactic acid, and to diminish ketonuria. This refractoriness to adrenalin is in contrast to the hypersusceptibility to insulin, small doses of which lowered the blood sugar and induced the symptoms of hypoglycemia, absent with

the spontaneous hypoglycemias. After ingesting dextrose the blood sugar curves have commonly risen to levels high in relation to the fasting values, but well below those observed in diabetes mellitus. The elevations have been prolonged, sometimes biphasic, and usually maintained for 3 or more hours. Even with relatively large amounts of dextrose glycosuria has not been observed. Other sugars gave similar curves and there has been no evidence of impaired tolerance for galactose and levulose. Tests for other types of impairment of hepatic function have failed to show significant deviations from normal. High normal or increased amounts of blood glycogen have been found, and while the data are not altogether in agreement, there have been no striking abnormalities of the diastatic ferments of the blood. Some observers have reported marked increases in the urinary excretion of diastase.

Discussion

With 15 proved and many probable cases reported since 1929 glycogen-storage disease probably is not a condition of extreme rarity. It is not impossible that milder types, transitory phases and other anatomical varieties of this disorder may occur. The elucidation of its mechanisms and characteristics requires the collaboration of the clinician, the chemist, the physiologist and the pathologist.

An immediate problem for the pathologist, suggested by the inadequacies of many of the methods used, is the proper handling of the tissues secured by biopsy or at autopsy. Perhaps it is superfluous to mention the desirability of chemical analyses, although we failed to appreciate it at the time of our autopsy. Satisfactory methods of preserving tissues and the use of proper controls of staining technique are essential to prevent erroneous conclusions, especially as to the absence of glycogen. While other fluids may be superior we have found that one of the best simple solutions for preserving glycogen in tissues is the one recommended by Bartelmez and Bensley [38] and by Burghgraeve.[39] This is a mixture of absolute alcohol and formalin, 9 parts to 1. Best's carmine is the most dependable stain and may be applied to sections embedded either in paraffin or in celloidin. Some artefacts seem to be inevitable but errors of interpretation may be avoided by using control sections known to contain glycogen, and sections treated with saliva for 30 minutes before staining.[38]

The lack of "normal" standards makes it difficult to interpret the significance of the moderate amounts of glycogen found in the non-hypertrophied organs. Of the many reports in the literature few attempt to correlate glycogen content with such factors as age, cause of death, interval between death and autopsy, and so on. Systematic studies prove that these factors are important.[40, 41, 42] On the other hand, the amounts of glycogen found in some of the enlarged organs are surprisingly large. Compared with Fränkel's[43] figures for normal skeletal muscle 0.5 to 2 per cent (the latter for the glycogen-rich muscle of the horse), the 9.39 per cent of glycogen found in the skeletal muscle of Pompe's patient by van Creveld[14] is amazingly high. The cardiac muscle of the same child contained 7.96 per cent of glycogen. Cardiac muscle is said to contain as much glycogen as skeletal muscle, possibly more, but loses it more quickly through postmortem glycogenolysis. The highest value we have found is 1.2 per cent for the heart of a dog after carbohydrate feeding.[43] The normal human kidney is supposed to contain very little glycogen. In diabetes mellitus Popper and Wozasek[44] found 1.64 per cent the maximum value for renal glycogen in their series (26 cases), while Schönheimer[7] observed nearly four times that amount, 6.53 per cent, in the kidneys of von Gierke's first patient. The figures 9.13 per cent,[14] 10.43 per cent,[7] and 14.2 per cent,[8] certainly represent large quantities of hepatic glycogen, although similarly high values are said to occur in other conditions. However, Popper and Wozasek's[44] analyses of 177 human livers yielded maximum values of 6.17 per cent after sudden death, and 8.50 per cent in diabetes mellitus. In analyses of the livers of 44 infants and children "made at the time of death" Burghard[45] reported a maximum of 5.2 per cent (diphtheria), and found only 5 with glycogen in excess of 2 per cent. In feeding experiments in animals, however, values above 20 per cent have been attained.[43, 46]

The identity of the cardiac and hepatic types of glycogen-storage disease is not proved, although it seems probable that the basic defect is a common one. The absence of frank cardiac enlargement does not exclude moderate involvement of the heart in cases of the hepatic type, such as was seen in the youngest child in the group.[8, 9] Many, if not all, of the children in the cardiac group had a similarly moderate involvement of the liver and kidneys. The strikingly earlier mortality in the second group may mean merely that the child

with cardiomegaly is more seriously handicapped and withstands infections poorly. We do not know whether or not with survival and seeming recovery there are residual metabolic or anatomical abnormalities. All that we know of the metabolic functions of the infants with cardiac disease is the fact that the ketonuria so characteristic of the hepatic type may be absent. On the other hand, even some of the "cardinal" symptoms of the hepatic type may be secondary, a statement suggested by the experiments of Junkersdorf.[46] By forced feeding with carbohydrate or with fat he was able to produce enlargement of the liver and hypoglycemia in young dogs. He regarded the hypoglycemia as hepatogenic, caused by overloading the liver cells with glycogen or fat, resulting in "mechanical" interference with their blood sugar-regulating functions. Obviously the separation of the basic from possible secondary metabolic defects would be facilitated by metabolic studies in non-hepatic types of the disease, if they could be recognized during life. Our Case 1 suggests a possible usefulness for biopsies of skeletal muscles in identifying such cases.

The removal of even a limited number of conditions from the unsatisfactory category, "idiopathic," encourages the investigation of other unexplained disorders of childhood. In view of the observations already made we might well examine critically the rare cardiac rhabdomyomas,[13] hypertrophic pyloric stenosis,[19] and the non-neurogenic diseases of skeletal muscle, especially the progressive muscular dystrophies. Obviously we must avoid the error of attributing too much significance to the presence of small amounts of glycogen, even in enlarged organs. It is equally obvious that conditions characterized by vacuolization should not be designated as "fatty" or "hydropic" without an attempt to identify the contents of the vacuoles. The search for glycogen in the tissues of the child with glycogen-storage disease is facilitated by its presence in such large quantities and by its resistance to spontaneous glycogenolysis. Furthermore, experience has shown that the search is not necessarily futile, even with tissues that have been improperly fixed or preserved for some time. Our own experience has confirmed the statement of Bartelmez and Bensley[38] that glycogen may be surprisingly well preserved in tissues fixed in Zenker's solution and embedded in celloidin.

It is essential to recognize that many symptoms and even combinations of some of the more unusual symptoms of glycogen-storage

disease may be shared by quite different diseases. This is true of certain types of hepatic cirrhosis, the peculiar syndrome described by Parnas and Wagner [47] and the famous case of Wilder and his coworkers.[48] Parnas and Wagner's patient had many symptoms resembling those of glycogen-storage disease, but eventually developed typical diabetes mellitus. Wilder's adult patient had persistent hypoglycemia and was refractory to adrenalin with respect to its blood sugar-elevating function. The point of special interest for us is that this refractoriness did not imply glycogen-impoverishment. At autopsy an insulin-secreting carcinoma accounted for the hypoglycemia, and the liver contained 8.25 per cent of glycogen. These examples merely serve to emphasize the fact that, at least for the present, the certain identification of glycogen-storage disease rests on the examination of tissues obtained at autopsy or by biopsy.

The most common assumption to account for the peculiarities of glycogen-storage disease has been that it represents a prolongation of fetal or infantile behavior with respect to the metabolism of carbohydrates. It is quite generally recognized that glycogen is abundant in the tissues of the fetus, even in tissues that contain little glycogen in the mature organism. The glycogenolytic capacity of fetal tissues is said to be slight. Furthermore, clinical evidence indicates that the carbohydrate-mobilizing mechanisms are poorly developed in the infant. In the proved cases of glycogen-storage disease morphological changes, which would implicate the endocrine organs and the nervous structures concerned in carbohydrate metabolism, have been lacking. Most observers have concluded that the defect probably lies in the affected tissues. Among the explanations offered are deficiency of ferments, binding of enzyme or protection of substrate, the presence of a heterotypical glycogen, and inhibition of hydrolysis by unfavorable reaction or by the accumulating products of hydrolysis. To understand the mechanisms we certainly need a more exact knowledge of normal carbohydrate metabolism, especially with respect to the mobilization of glycogen, than we have today.[49] The fact that glycogen-storage disease occurs as an infantile disorder, probably congenital, and possibly familial, invites comparison with the interesting class of metabolic diseases characterized by Garrod [50] as "inborn errors of metabolism." Interestingly most of these congenital or familial metabolic disorders are attributed to abnormal enzymatic functions.

Summary and Conclusions

Glycogen-storage disease (*Thesaurismosis glycogenica*, von Gierke) is a disorder of infancy and childhood, possibly familial, and affecting both sexes. It is characterized anatomically by organ enlargement and the storage of glycogen chiefly, but not exclusively, in the enlarged organs. Two main types have been delimited, glycogenic hepatomegaly or hepatonephromegaly, and glycogenic cardiomegaly. The affected organs may attain great size and may contain glycogen in unprecedented amounts. One peculiar feature is delayed postmortem glycogenolysis and the search for glycogen has been successful in spite of adverse circumstances and imperfect techniques. In only a few instances has glycogen-storage been associated with degenerative or proliferative changes. At least in the hepatomegalic type clinical studies have seemed to point to an impairment of the mechanisms concerned in the mobilization of glycogen and the regulation of the blood sugar. Less is known of the cardiomegalic type.

We present the clinical and autopsy studies of 3 proved cases and 1 probable case of cardiomegalic glycogen-storage disease. The weights of the hearts were 3.3 to 7 times normal, and the myocardial fibers were enlarged, vacuolated, and (in 3 cases) filled with glycogen. It is probable, though proved only in 1 case, that the liver and kidneys were affected similarly but to a lesser degree. In 2 cases the skeletal muscles showed an unusual type of vacuolar degeneration, and in 1 of these the vacuoles contained much glycogen. This report brings the total number of proved cases to 15.

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DESCRIPTION OF PLATE

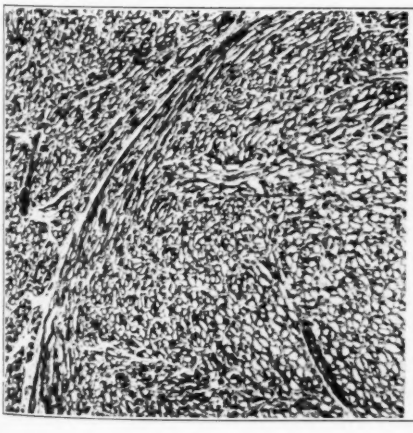
PLATE 141

FIG. 1. Case 2. Glycogenic cardiomegaly. This heart weighed 260 gm., about seven times normal for the age, 8 months. The increase in size was due to general enlargement of the organ, without dilatation. Photograph 10 per cent larger than actual size of the fixed heart.

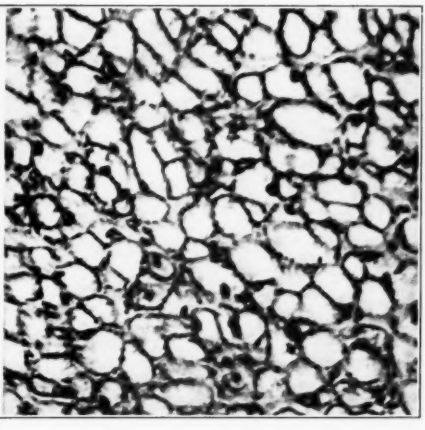
FIG. 2. Case 1. Glycogenic cardiomegaly. Photomicrograph demonstrates the lacy appearance of the myocardium resulting from the almost universal vacuolization of the muscle fibers. Mallory's phosphotungstic acid hematoxylin stain. × 75.

FIG. 3. Case 1. Photograph of the large myocardial fibers cut obliquely or transversely. Mantles of myofibrils surround seemingly empty axial spaces. In sections stained with Best's carmine these vacuoles were more or less completely filled with glycogen granules. Mallory's phosphotungstic acid hematoxylin stain. × 310.

1



2



3

Humphreys and Kato

Glycogen-storage Disease

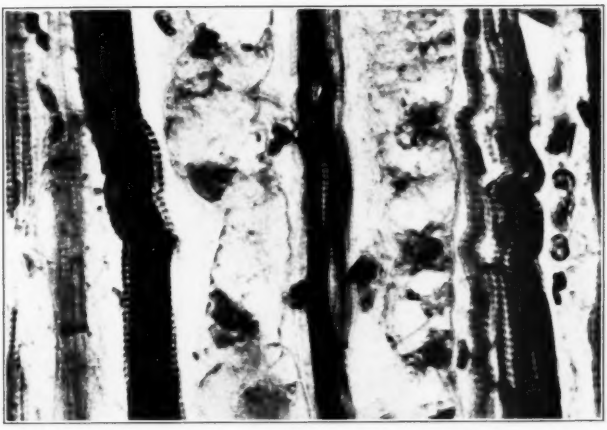
PLATE 142

FIG. 4. Case 1. Skeletal muscle. Photomicrograph of a single swollen muscle fiber cut lengthwise. The bulging sarcolemma contains scattered degenerating nuclei and granular and fibrillar débris. In carmine-stained sections some sheaths of this type were filled with coarse glycogen granules. Mallory's phosphotungstic acid hematoxylin stain. × 550.

FIG. 5. Case 1. Skeletal muscle. Photomicrograph shows large fibers of the type seen in Fig. 4, alternating with compact, deeply-stained normal fibers and with others whose sparser fibrils appear as if teased apart. Mallory's phosphotungstic acid hematoxylin stain. × 550.

FIG. 6. Case 1. Skeletal muscle. Photomicrograph of muscle fibers cut transversely to show various degrees of vacuolization and degeneration. The nearly empty sheaths are cross-sections of fibers of the type seen in Fig. 4. The retraction of some of the sarcolemma sheaths may have been caused by shrinkage. Mallory's phosphotungstic acid hematoxylin stain. × 550.

4



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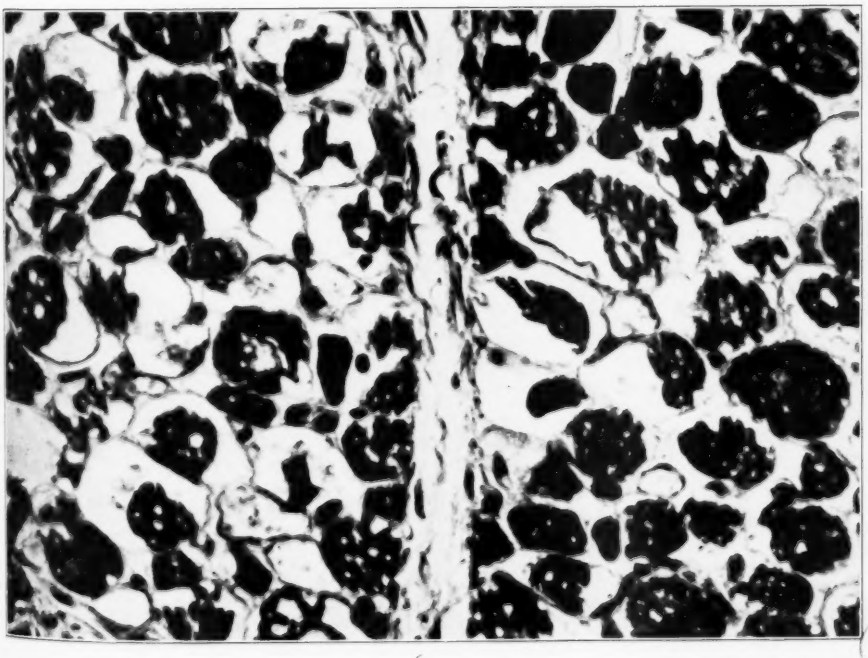

6

ALTERATIONS IN MINERAL CONSTITUENTS OF ANTERIOR HORN CELLS IN EXPERIMENTAL POLIOMYELITIS *

W. E. PATTON, M.D.

(From the Anatomical Laboratory, Washington University School of Medicine, St. Louis, Mo.)

The observations herein reported form part of a comprehensive study of the reactions of cells to viruses. The technique is that of microincineration already utilized in this laboratory for investigation of the alterations provoked by the salivary gland virus of guinea pigs (Scott [1]) and the viruses of rabies (Covell and Danks [2]), fowlpox (Danks [3]), yellow fever (Cowdry [4]) and herpes (L. E. and E. J. Rector [5]). The method, as introduced by Policard [6] and modified by Scott,[7] makes possible the exact topographical localization of the mineral constituents within a single cell, after the removal of its organic constituents by a process of burning. It is easily possible to control the observations by comparing the ashed section with the next following stained section of the same cell. When one considers the important rôle played by inorganic salts in cellular permeability and the colloidal organization of the cytoplasm the importance of accurate data on changes in their distribution is evident.

MATERIAL AND METHODS

Material was obtained from the spinal cords of 43 monkeys experimentally infected with poliomyelitis by intracerebral injection of a virulent cord emulsion. The animals were sacrificed at various periods of nerve involvement ranging from slight fibrillary tremors to complete prostration. Tissue from the spinal cords of 10 normal monkeys was used as control. Pieces 3 to 8 mm. thick were removed from the cervical (fourth to sixth) and lumbar (third to fifth) enlargements and immediately fixed in 10 per cent formalin in absolute

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alcohol, Zenker's fluid, and Zenker's fluid without acetic plus 10 per cent formalin. In some cases Zenker's fluid was applied by perfusion through the blood vessels.

The absolute alcohol-formalin fixative was recommended by Scott [8] to prevent solution or displacement of inorganic salts in cells prepared for microincineration, while at the same time preserving the cytological structure. Full details of the technique of microincineration are given by Scott.[9]

All sections were cut at 4 microns, since it was found that in thicker specimens the nerve cells showed a marked tendency to shrinkage during incineration. Even at this thickness the outlines of the smaller cells may be contracted in the ashed preparation, but the larger cells reproduce almost exactly the size and shape of the control stained section, or show only such variation as is seen between the outlines of a nerve cell in two consecutive sections. The phenomenon of shrinkage may give rise to a heavy ash about the cell membrane, which may lead to the erroneous impression of a peripheral concentration of mineral matter. In the perfectly prepared specimen such an appearance has not been noted. It was found that slides incinerated on days when the humidity was unusually low presented a clearer picture than those prepared when the air contained more moisture. Even during the short time required to cool a slide the ash particles may sometimes absorb enough moisture to cause a yellowish to dark brown coloration which may be confused with the amber or reddish tint so characteristic of iron-containing tissues. It may also resemble the appearance of sections in which carbon remains after incomplete incineration. The color alteration due to moisture, however, disappears immediately after heating over a Bunsen flame and may be attributable to a change in the refractive properties of the ashed particles brought about by the deposition of a thin film of water on their surface.

After the other fixations the sections were cut 5 microns thick and stained with hematoxylin and eosin, Giemsa's stain, erythrosin-azur or phloxine-methylene blue. The purpose of this was to have at hand an abundance of material prepared by ordinary routine methods to illustrate the well known reactions of the cells to the virus for comparison with the results secured by the new incineration technique. But for making the detailed topographical comparison serial sections of the formalin-alcohol preserved tissues were cut, alternate ones

being mounted with double filtered absolute alcohol (for incineration) and with egg albumin (for staining). Observations were limited to those involving identification of the same cell in consecutive sections after incineration and staining. This was done by noting its position in relation to the outline of the anterior horn, its proximity to such landmarks as blood vessels, and the similarity of cell outlines in the two sections.

For observation of the specimens two Zeiss binocular monobjective microscopes were set up side by side, one equipped with the usual Abbé condenser for examining stained slides, the other with a Zeiss cardioid condenser for dark-field study of the incinerated preparations. The lens systems on the two microscopes were identical. This permitted accurate judgment of shrinkage, distortion and other changes. As a light source a large Spencer lamp (No. 394) fitted with a Projection Mazda, 500 W, 115 V, General Electric bulb and without a daylight filter was used. When one becomes accustomed to the yellowish light provided by this arrangement it is found that color discrimination is in no way interferred with. To guard against the possibility of confusion from structural color (Mason[10]) the dark-field observations were checked with incident light from a lamp adjusted in such manner that the beam fell directly on the specimen. This arrangement, of course, could not be employed when an ordinary immersion lens was used. It was found that some of the bluish color in certain structures was not so marked with direct lighting, giving some support to the theory that this might be due to a refractive phenomenon. It cannot be said, however, how much of the color loss is attributable to the distinctly inferior lighting which is obtained with incident illumination. If, as Mason believes to be true for similar materials, the bluish color of this type of preparation is due to a dispersion phenomenon it is still significant since it indicates a definite localization of cellular substance which differs either physically or chemically from that in adjacent areas.

Observations

Attention was confined to the large somatic motor cells of the spinal cord in an effort to determine the sequence of changes in neural constituents and to characterize each alteration as accurately as possible.

Normally these cells contain rather less inorganic material than the Purkinje and spinal ganglion cells described by Scott.[8] The cytoplasmic ash is made up of an extremely finely divided and evenly distributed dust-like residue usually of a more or less bluish hue, as already reported (Patton [11]). In this background are found small masses of heavier, flat white material, which in distribution and shape correspond to the Nissl bodies, as seen in the immediately following or preceding control-stained section of the same cell. Close study shows that these masses have a faint but distinct yellow color — a property that may indicate the presence of iron. The nuclear membrane gives a denser and more pronounced ash than the cell membrane. The nucleoplasm does not leave a uniform fine residue like the cytoplasm. Instead its background has a vacant appearance. Set here and there are a few rather definitely outlined clumps of yellowish residue. These probably remain from the particles of iron-containing material in the nuclei featured by Nicholson.[12]

Three stages, which merge the one into the other, may be recognized in the alterations resulting from virus action. These are represented by the three pairs of figures on the plate. The figure on the right of each pair represents the mineral skeleton of a single cell cut in section and viewed in the dark-field, while the one on the left is the immediately adjacent section of the same cell, not incinerated but colored in the usual way with hematoxylin and eosin.

1. *Stage of Edema* (*Figs. 1 and 2*): The first change is what Nissl called "acute swelling." When examined in stained preparations the outlines of the cells are seen to be rather rounded and swollen, but the cellular membranes remain intact. The Nissl bodies are lost by "chromatolysis," first near the nuclei but later throughout the cytoplasm. The cytoplasm becomes very pale and looks uneven. The nuclear changes are noticeable soon after the loss of Nissl bodies and represent the first signs of the severe cell injury, which ultimately ends in death. The nucleus, like the cell body, first becomes swollen but later tends to shrink, with a wrinkling of the nuclear membrane. It may be displaced and occupy an eccentric position. The tendency is toward a peripheral migration of the basophilic chromatin, which remains plastered on the membrane; but some of it may still be seen as small, deeply staining, beady granules or as stellate, irregular masses in a net attached like a spider's web to the nuclear mem-

brane. It is in cells during this stage that the "nuclear inclusions" of Covell [13] and Hurst [14] first make their appearance. A large one is represented above and to the right of the spherical nucleus and a smaller one above and to the left in Figure 1. They are sharply outlined, spherical globules ranging in size from minute specks to bodies of 3 to 4 microns in diameter and are amphophilic in staining property, but may be made to appear acidophilic by varying the degree of differentiation. Sometimes the inclusions seem to have a darker center with a lighter periphery — probably a refraction phenomenon, because the ring disappears on slightly altering the focus of the microscope. On no occasion has a halo been observed, such as is described by Nicolau and Galloway [15] for the nuclear inclusions in Borna disease. These bodies may be numerous within a single nucleus. As many as 8 or 10 have been counted in one cell. In general it may be said that they tend to be smallest when they are most numerous and *vice versa*. Eventually, in every case, the nucleus becomes completely filled with the granular amphophilic material, in the midst of which an unaltered nucleolus may be distinguished by its basophilic staining properties.

Microincineration shows that there is a gradual and parallel diminution in the amount of mineral matter (Fig. 2). The cytoplasm is represented by a thin, finely divided, evenly distributed ash similar to that left by the non-stainable cytoplasm of the normal cell, but the residue of the Nissl bodies does not appear. The nuclear membrane is rendered more prominent by the peripheral migration of basophilic chromatin and appears as a heavy white line studded with clumps of ash. This observation lends additional weight to Scott's [16] statement that most of the mineral matter of the nucleus is located in the basophilic chromatin. The inclusion bodies are too small to extend through two sections but at this stage in development they yielded a small amount of ash. A few of the yellowish ash masses are identifiable and the large, conspicuous nucleolus yields a dense, flat white residue.

2. *Stage of Increase in Mineral Matter* (*Figs. 3 and 4*): Up to this point the nucleus and cytoplasm have shown fairly simultaneous changes, but here the parallelism ceases. When both continue they pursue a similar sequence of alterations in every case but either may outstrip the other in the rate at which they occur. Thus we may find a nucleus in the last stages of degeneration while the cytoplasm

shows only the earliest alteration, a condition that causes considerable confusion, but no inconsistency of the picture.

In stained sections the pallid cytoplasm begins to be marked here and there with clouded areas, the alteration spreads, and soon the entire cell body takes on a deeper color with basic dyes — darker even than the nucleus. The outlines of the cell are sharp and well defined, and although no marked shrinkage may be noted, tend to approach those of the normal neurone. As the hyperchromia develops the cytoplasmic contents, colored in this way, become a mass of deep blue-stained granules, translucent, thickset, filling the entire cell and all its processes. Vacuoles may appear. The granules do not remain unaltered long; they tend to coalesce, resolve or concentrate themselves into small, dense, dark beady masses which are seen against a spongy reticulum (Fig. 3). When viewed with high magnification and strong illumination the larger ones assume the appearance of ringlets, which appearance fades with a slight change in focus. Such rings in other cells were pointed out by Nissl as being the chief characteristic of what he called "*schwere Zellveränderung*," although Spielmeyer observes that he has seen them occasionally in man and less frequently in animals. The possibility that these granules might be pigment was considered. Their size, shape and distribution, as well as their constant presence in alcohol-fixed tissues, tends to rule out pigments of the fatty type. Sections stained with methylene blue alone, in which the granules took the stain intensely in every instance, were studied. As a final check serial stained and unstained mounted sections were examined. Cells that showed the granules in the colored section showed no trace of yellowish coloration in the unstained preparation.

The nucleus, last described as being filled with minute spherules of varying size, meanwhile undergoes some alterations. Without variation in amphophilic staining qualities the spherules coalesce and the nucleus becomes a contracted, partly homogeneous mass. Within it may be seen one or more round bodies of somewhat darker material, one of which may represent the nucleolus, others remains of the nuclear material which have not melted into the mass. Compare the nuclei in Figures 1 and 3.

It is in cells at this stage of degeneration that neuronophagia is first observed. The entire cytoplasm and its formed constituents seem to be engulfed by the phagocytic invaders. But some de-

generating cells escape phagocytosis, for some of them are observed even in the last stages of degeneration without even so much as a marked satellitosis.

Microincineration of such cells reveals a striking change (Fig. 4). The cytoplasmic ash, which formerly had been less in amount than that seen in the normal nerve cell, is increased with the onset of the cytoplasmic clouding. It continues to increase with the granular change, reaching a maximum at the stage in which the deeply stained, shotty, basophilic granules are present. When seen at low magnification attention is immediately attracted by these brilliantly refractile clumps of ash within the anterior horn. Close inspection gives the impression that this dense cytoplasmic ash is piled on the slide. The cell outlines are distinct; there is little shrinkage. The ash shows from the first an increased tendency to arrange itself in clumps, and is composed of coarsely granular, white, glistening, glassy particles. The cell processes share in the increase of inorganic material and are represented as brilliant streaks in the gray matter.

The nuclear picture may be obscured by the overlapping of a rim of cytoplasm or by the scattered bits of ash representing the basophilic chromatin. Some cases may be found, however, in which the cytoplasmic mineral is excluded. Such sections indicate that the amphophilic homogeneous material contains a small amount of inorganic matter in the form of a finely divided, film-like ash, flat white or bluish, and evenly distributed. On some the chalky residue of the nucleolus is seen, not strikingly different from that found in the normal neurone. This is the last stage in which the nucleolus can be identified, in either stained or incinerated sections. What its subsequent fate may be, whether it is absorbed, destroyed by rhexis or extruded, could not be determined.

The neuronophagic foci leave an ash dependent on the amount of cytoplasmic mineral matter of the cell attacked. If a granular hypermineralized cell is being destroyed the incinerated focus presents the brilliant ash characteristic of such cells. Apparently the process of neuronophagia is carried on without change in inorganic constituents. The scavenger cell can incorporate and, as it were, digest the entire abnormal mineral content of the dying neurone.

3. *Stage of Necrosis (Figs. 5 and 6):* Comparison of Figures 5 and and 6 with 3 and 4 will demonstrate how marked are the changes

that occur slowly and by imperceptible gradations in the living animal.

Study of stained sections indicates that as the cells become acidophilic the cytoplasmic granules may be phagocytosed, extruded, or disappear by some not easily explainable process, perhaps by lysis. Similar granules may sometimes be seen in polymorphonuclears near the site of the lesion or extracellularly in the gray matter, especially near necrotic cells. The dead, acidophilic cytoplasmic débris left behind usually contains numerous large vacuoles, which are not illustrated in Figure 5.

The amphophilic and almost homogeneous nucleus becomes more and more acidophilic. This alteration in reaction may occur in cells still containing granules, but generally appears approximately coincident with their disappearance. The final nuclear picture, then, is that of a rounded, acidophilic, clear homogeneous lump separated from the cytoplasmic remains by an empty shrinkage space.

Cells of this type are usually removed by neuronophagia. Others may be seen in which the cytoplasm melts away by a lytic process. In these the acidophilic cytoplasm becomes progressively more vacuolated until it is represented by a delicate web of liquifying material. In such cells the nucleus persists to the end and sometimes may be seen in a pericellular space in which no trace of cytoplasm remains.

In incinerated preparations the cells in this final stage are easily recognized. Compare Figure 6 with Figure 4 of the preceding mineral-rich condition. When for some unknown reason the cells are not phagocytosed like their neighbors the excess of mineral matter is reduced in some other way. They retain, however, in death more than they possess in the first stage of the reaction (Fig. 2). The ash is distributed fairly evenly in the cytoplasmic area but is lacking in the spaces occupied by the vacuoles mentioned. The original location of some of these vacuoles is marked by the absence of ash. The hyaline necrotic nucleus leaves little or no ash.

Another type of cell change is frequently found in poliomyelitic cords, although it is not as characteristic as the process just described. It is more often seen in advanced cases, in which meningeal infiltration and perivascular cuffing are maximal. In appearance the cells resemble closely the pathological forms described by Nissl as characteristic of ischemia. They display a thin and chromophobic

cytoplasm, with markedly contracted outlines. The nucleus stains dark blue with hematoxylin and eosin and tends to assume a characteristic triangular shape when cut in section. The nucleolus is swollen. The disposition of inorganic components in these cells provides an interesting contrast. The nucleus exhibits a heavy, powdery, refractile ash, while the cytoplasm is represented by a deposit so sparse that it can scarcely be appreciated in the incinerated specimen. It is evident that a migration of inorganic substance from the cytoplasm to the nucleus has taken place, an exact reversal of the process described above, in which the mineral matter of the cytoplasm increased at the expense of the nucleus.

Discussion

As in other injuries and infections of the nervous system, edema occupies a conspicuous place in the pathology of poliomyelitis. It is usually accompanied by an acute swelling of the cells, which disappears as reaction to the injury subsides. Somewhat similar appearances may be produced by the intravenous injection of considerable quantities of water (Weed and McKibben [17]). With a decrease in ionic concentration of the fluid environment of the cell one would expect to find a diffusion of water into the cytoplasm and a diffusion of salts outward. The slight diminution of cytoplasmic mineral constituents demonstrated by microincineration during the stage of swelling may thus be due both to loss of cytoplasmic salts and to dilution. The alteration may not be considered permanent, since it is but a stage in many diseases in which complete recovery ordinarily occurs, and it may be reversed by the injection of hypertonic solutions.

The earliest indication of severe injury is seen in the nucleus with the appearance of the bodies described by Covell [13] and by Hurst,[14] who suggested that they might be specific for the disease. Such bodies have also been observed by Schultz [18] and by Stevenson,[19] the latter author being more conservative in his interpretation, stating that they "seemed to be nothing more than a clumping of the intranuclear network." They appear in the drawings of Walter [20] and of Wickman [21] who, however, did not mention them as being specific. Wolf and Orton [22] recently reported finding similar "intranuclear inclusions" in the spinal motor cells of patients dying of twenty-five

different diseases. The results of the present study indicate that they merely represent one stage in the total process of cellular degeneration. It is difficult to postulate the origin of the inclusion material. It might consist, as Wolf and Orton have suggested, of the vaguely defined substance known as "acidophilic chromatin," in which case it would represent an increase of a normal nuclear constituent. On the other hand, their mineral composition would indicate that these bodies represent an alteration of the nuclear ground substance by diffusion into it of some of the inorganic matter from the basophilic chromatin, which is gradually lost as its staining properties change from basophilia through amphophilia to acidophilia. Whatever it may be composed of, there is little doubt that the substance is formed with considerable rapidity and in large quantities, since it soon comes to fill the entire nucleus. The rate of its formation does not always parallel the rate of change in staining properties, a fact that may account for the occasional appearance of an acidophilic "inclusion body."

The granular stage of degeneration is one that has been overlooked by most investigators. Rissler [23] described a "granular clouding of the cytoplasm" as one of the earliest evidences of poliomyelitic injury, and Kraus and Gerlach [24] found certain deeply staining granules which they thought might be specific for poliomyelitis. Later, however, Gerlach and Kress [25] discovered them in other pathological states and decided that, although they were not unique to poliomyelitis, they certainly represented specific products of the action of a virus on a cell. They concluded that the granules were composed of lipofuscin (Lubarsch) or other similar pigment. In view of the fact that their inorganic content is high, and that most pigments are of almost purely organic composition, it seems unlikely that their conjecture could be true.

The frequent oversight of this stage of degeneration may be attributed to the briefness of its existence during the earliest stages of involvement (later stages are more often studied, especially in human cases) or to the difficulty of recognizing it in routinely stained sections. In the microincinerated preparations, on the other hand, the appearance of the granular cells is so striking that it cannot escape notice, and the change which renders them so obvious constitutes, from the microchemical standpoint, the most interesting phase of the entire process. Obviously, nuclear mineral matter enters the

cytoplasm but not in sufficient quantities to explain the increase. Some must be derived from without, from tissue fluid, blood or other sources by a necrobiotic attraction, a final vital action of the cell, after which it proceeds to complete disintegration. No explanation can be offered of the fact that some cells in this particular stage are phagocytosed, while others are not.

The recognition of the granules is important for another reason: it serves to classify the cytopathology of the disease. If the series of cell changes here described be compared with Spielmeyer's [26] account of Nissl's fundamental change, which he termed "*schwere Zellveränderung*," it will be found that they agree closely. If the same type of destruction has been observed in various diseases it would seem that the recognition of poliomyelitis by a single stage of the process would be impossible, and that the effects of the virus on the cell were anything but specific. The coincidental formation of inclusion bodies, which differ from the cell changes in other diseases showing this type of degeneration, would appear unlikely.

A problem of importance in any study of poliomyelitis injury is the determination of what might be called the threshold of mortality, or that amount of damage beyond which no recovery may occur. That some degree of normal function may return after the initial paralysis has long been known from clinical observations. O'Leary, Heinbecker and Bishop [27] showed that in the causation of paralysis we have to deal with some factor affecting the conductivity of the cell body. Covell [28] found that at the time their tests were made cell destruction was far advanced, so that the succession of cell changes described herein had probably already taken place. He also presented evidence that some of the partially destroyed cells might recover. The transient nature of acute cell swelling might suggest its relation to transient paralysis, but we know that this change may also occur in states in which no paralysis is present (meningitis, uremia, and so on). Unless, then, the paralysis is due to some specific action of the virus on the cell (which seems unlikely, considering the resemblance of the reaction to that seen in other diseases) we must conclude that it occurs some time after the appearance of nuclear changes. The results of this study indicate that the process of cell death is a complicated one. Osterhout [29] found the same to be true of planarians and was able to define a point beyond which complete recovery was impossible. Perhaps the same principle may be ap-

plied to nerve cells, in which case permanent loss of function would probably coincide with the stage of hypermineralization, since this stage, one would judge from the accounts of other authors, is approximately the time of destruction of the neurofibrillar network.

Summary

The principal type of nerve cell destruction in poliomyelitis involves three stages: (1) edema, with acute swelling of the cell and diminution of its inorganic content; (2) granulation, with hypermineralization; and (3) acidophilic necrosis, with dimunition of mineral constituents.

Note. The author is indebted to Dr. Gordon H. Scott and to Dr. E. V. Cowdry for help and encouragement.

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DESCRIPTION OF PLATE

PLATE 143

All figures are camera lucida drawings of anterior horn cells from monkeys experimentally infected with poliomyelitis. The figures at the left are drawings of control sections stained with hematoxylin and eosin. The figures at the right are microincinerated sections of the same cell. Material figured was fixed in absolute alcohol-formalin.

FIG. 1. Anterior horn cell from the cervical cord of a monkey experimentally infected with poliomyelitis showing acute swelling and the earliest stages of nuclear alteration. Two spherules of amphophilic material (the so-called inclusion bodies) are shown. The nuclear membrane is encrusted with basophilic chromatin, although some of this material still remains dispersed within the nucleus.

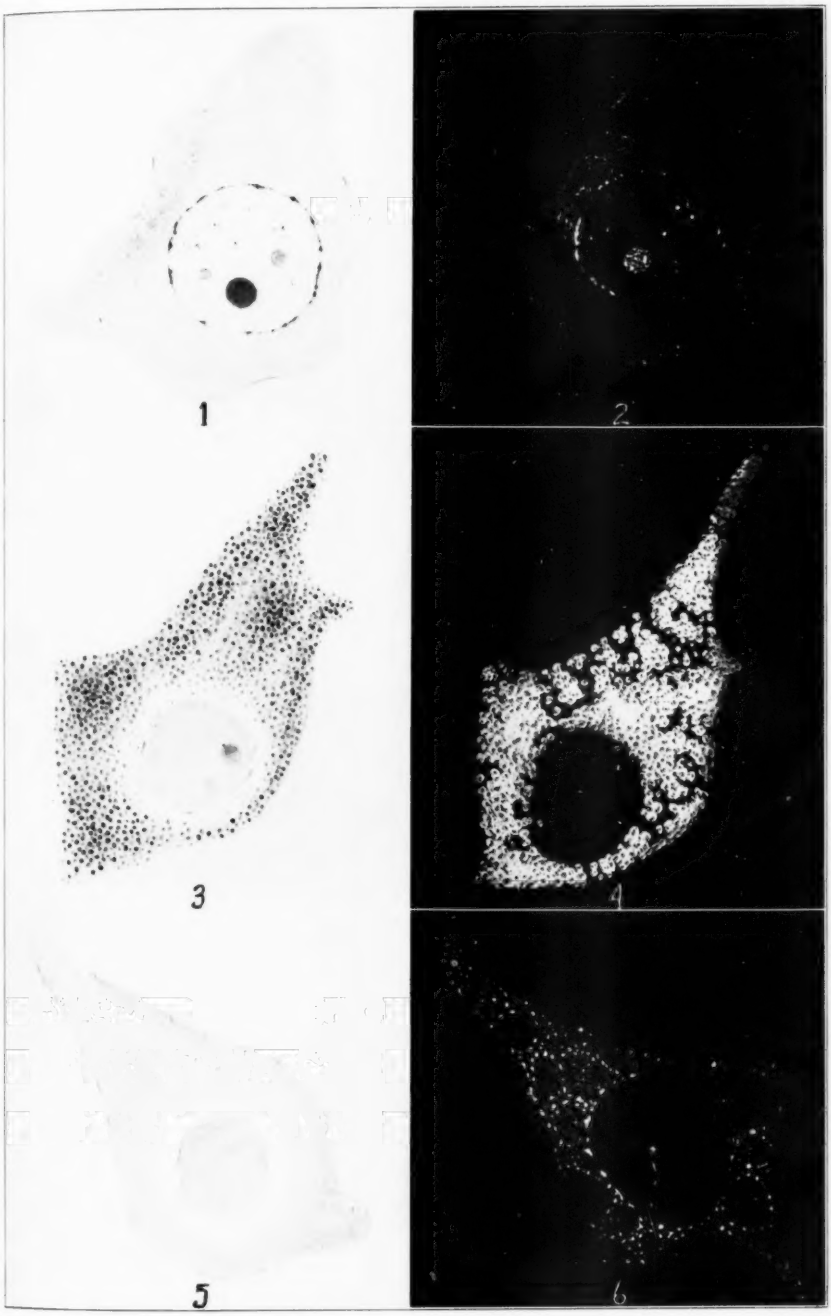
FIG. 2. Microincinerated preparation of a serial section of the same cell showing diminished cytoplasmic ash and increased density of nuclear membrane.

FIG. 3. Cell in the granular stage of degeneration. The nucleus is hyaline, acidophilic, and contains two round masses which take a deeper stain than the remainder of the nucleus.

FIG. 4. Microincineration of a section of the same cell as pictured in Fig. 3 showing the extremely heavy ash deposit left by the cytoplasm. The nucleus at this stage is practically ash-free.

FIG. 5. Acidophilic necrotic cell in which neuronophagia has not taken place. The cytoplasm exhibits a spongy texture and shows no cell membrane.

FIG. 6. Microincinerated section of the cell shown in Fig. 5. The ash is webby in appearance and is glossy, though not highly refractile.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INTRANUCLEAR INCLUSIONS IN THE SALIVARY GLANDS OF MOLES *

ELEANOR J. RECTOR, M.S., AND L. E. RECTOR, B. S.

(From the Anatomical Laboratory, Washington University School of Medicine, St. Louis, Mo.)

Intranuclear inclusions have been described in the salivary glands of guinea pigs, rats and humans, but only in guinea pigs has it been proved that they are caused by a filterable virus (Cole and Kuttner [1]). We have noted [2] their presence in a fourth species, the common mole. This observation was made in the course of a brief survey, suggested by Dr. E. V. Cowdry, of the distribution of intranuclear inclusions in wild animals in the absence of distinctive clinical symptoms. In this paper we shall describe these inclusions in moles, mention our attempts to transmit a virus and compare the properties of the inclusions in the salivary glands of all four species.

MATERIAL

We list the other animals we examined, as well as the moles, because negative observations may be of some interest to investigators making more complete surveys. They were:

1	Evans king snake (*Lampropeltis calligaster*)
1	Red headed skink (*Eumeces quinquelinaetus*)
1	Electric eel (*Electrophorus electricus*)
1	Bull frog (*Rana catesboana*)
2	Rats (*Mus decumanus*)
2	Mice (*Mus musculus*)
1	Gopher (*Spermophilus franklini*)
14	Moles (*Scalops aquaticus*)
1	Bat (*Myotis subulatus*)
1	Flying squirrel (*Glaucomys volans*)
4	Opossums (*Didelphis virginiana*)
11	Monkeys (*Pithecus rhesus*)
1	Gray cheeked mangabey (*Cercocebus albigena*)
1	Tapir (*Tapirus Indicus*)

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Our thanks are due to Dr. A. M. Lucas for the first moles showing inclusions, and to Mr. George Vierheller, Director of the St. Louis Zoological Gardens, for the mangabey and tapir.

Careful autopsies were made of each animal and specimens were taken from a wide variety of tissues. Zenker and Zenker-formalin fixatives were used in all cases and the sections were colored with hematoxylin and eosin and by the Giemsa stain. Special microchemical tests were applied to the inclusions in the moles and attempts to transmit a virus were made which will be described later.

Observations

Figures 1–6 illustrate the appearance of the intranuclear inclusions in the salivary glands of moles. They were found in all of the 14 moles examined. Careful search in other tissues failed to reveal any. The moles weighed about 150 gm. and were presumably adults. The series did not contain any very young animals. The sections were 5 microns thick. On the average 1.8 inclusions were found per sq. mm. of section. The maximum number was 9 per sq. mm., while in 2 animals the inclusions were very rare, being seen only after intensive study of many serial sections.

The altered cells are usually situated in the terminal tubules of the serous portions of the glands and more rarely in the mucous parts (Fig. 5). None occur in cells that can be definitely classified as duct cells but they are rather more numerous near the ducts. Many appear to be shoved off into the lumens of the tubules (Fig. 6).

An accompanying tissue reaction was noted in 8 animals. It consisted of a slight perivascular and interstitial lymphocytic infiltration. But the extent of the infiltration was not proportional to the number of affected secretory cells. Neither did the location always correspond. In some cases many cells possessing inclusions were observed in the absence of infiltration. Two nematodes were noted. The first, belonging to the genus *Capillaria*, was found in the epithelium of the tongue of 6 moles; and the second, apparently a member of the genus *Porrocaecum*, was observed in the salivary glands of 7 moles. For the identification of both of these we are indebted to Dr. E. W. Price of the Bureau of Animal Industry. There was no sharp correlation between the incidence of the parasites and the inclusions, but it was noted that in several sections the number of inclusions was

definitely higher near the *Porrocaeca*. The parasites were usually well walled off by connective tissue and were frequently accompanied by a mild degree of cellular infiltration, consisting chiefly of lymphocytes. The tissue reaction seemed to bear no constant relation to either the inclusions or parasites. In one mole there was, as might be expected, a heavy accumulation of eosinophile leukocytes near a *Porrocaecum*.

An outstanding feature of the inclusion-containing cells and nuclei was marked hypertrophy. The diameter of the altered cells averaged about 15 microns — approximately 1½ times the diameter of the normal cells. The extent of enlargement for both serous and mucous cells was remarkably uniform, as indicated in Table 1.

TABLE I

Measurements in Microns of Inclusion-containing Cells in the Salivary Glands of Moles

Cells	Diameter measured	Range	Mean	Median	Mean deviation on mean	Standard deviation
200 inclusion-laden cells in the mixed portion of the gland	Cellular	10.00	15.37	15.06	1.57	2.13
	Nuclear	9.34	10.14	10.67	1.56	1.83
	Inclusion	6.88	6.12	5.69	1.01	1.29
25 inclusion-laden cells in the mucous portion	Cellular	8.13	16.85	16.77	2.03	2.34
	Nuclear	6.25	12.05	12.03	1.75	2.13
	Inclusion	2.50	7.05	6.36	0.81	0.98

The mean is the mathematical average of the measurements obtained by dividing the sum of the diameters by the number. The median is the middle number in the series of numbers arranged in the order of magnitude. The fact that the mean and median are so nearly the same indicates a central tendency. However, one can easily imagine that a distribution in which there is an equal clumping at each end of the range might easily give a median and a mean of almost equal value. To minimize this possibility of error the "mean deviation on the mean" and the "standard deviation" were calculated. In the "mean deviation on the mean" we have a figure showing the average distance of all members of the series from the mean, or average, of the series. This is remarkably small and gives a truer indication of a central tendency. The "standard deviation" is

a measurement which, when marked off on both sides of the mean, will include two-thirds of the cases involved, when the cases have been arranged in the order of magnitude. Interpretation of this central tendency is difficult. It may be that the cellular response, whatever its nature, has reached a kind of end point characterized by relative uniformity in degree of hypertrophy. It is possible also that the early stages of hypertrophy, so rare in our specimens, seem to be lacking because they may be passed through quite rapidly; and that final stages of further hypertrophy or disintegration are absent, because the cells may be cast out through the ducts at this particular stage. Moreover, the infrequency of early stages may indicate that the process is no longer an active one.

The intranuclear inclusions themselves show a parallel uniformity in size. The range from the largest to the smallest was slight. Occasionally they seemed to be paired, or subdivided (Figs. 2 and 4). Their outlines were somewhat irregular. The centers were denser than the periphery. They were definitely basophilic, only rarely showing any tendency towards acidophilia — a property that we do not think can be attributed to unusual fixation or some deviation from standard technique.

The intranuclear inclusions exhibit marked Feulgen and masked iron reactions. Mucicarmine and Millon's reagent gave inconclusive results. Margination of chromatin on the nuclear membrane was not very noticeable. This may be correlated with the retention by the inclusion of basophilic properties (thymonucleic acid and masked iron). The nucleolus was recognizable in some cases either applied to the inclusion or between the latter and the nuclear membrane. A clear thin halo of chromophobic nuclear material is invariably interposed between the inclusion and the nuclear membrane and is very typical.

In the cytoplasm of most cells 10 to 30 small, basophilic inclusions were clearly visible, closely resembling those described in detail in the guinea pig's submaxillary glands by Pearson.[3] They accompanied approximately 40 per cent of the nuclear inclusions and occurred in both serous and mucous types (Figs. 1–5).

Many attempts were made to demonstrate the existence of an active virus in the salivary glands, but saline emulsions injected intracerebrally, subcutaneously and intraglandularly into rats, mice, rabbits and young guinea pigs proved futile (Table II). The animals

that died in a few hours did so as a direct result of injury to the brain when the injection was made and not from the action of a virus. Those that survived until they were sacrificed showed no signs of dis-

TABLE II

Attempts to Pass Virus

Mole No.	Inoculum (saline emulsion)	Recipient animal	Injection	Result
4	Fresh tissue	Rabbit	Intracerebral	Died — 6 hours
4	" "	Young guinea pig	Subcutaneous	Killed — 30 days
5	" "	Rabbit	Intracerebral	Died — 6 hours
5	" "	"	"	Died — 12 hours
5	" "	"	"	Killed — 14 days
5	" "	Young guinea pig	"	Died — 12 hours
4, 5, 6	Glycerin preserved tissue	White mouse	"	Killed — 35 days
4, 5, 6	" " "	" "	"	Died — 3 days
4, 5, 6	" " "	" "	"	Killed — 38 days
4, 5, 6	" " "	White rat	"	39 days
4, 5, 6	" " "	" "	"	39 days
4, 5, 6	" " "	" "	"	39 days
4, 5, 6	" " "	Young guinea pig	"	38 days
4, 5, 6	" " "	" " "	"	38 days
4, 5, 6	" " "	" " "	Submaxillary gland	20 days
12	Fresh tissue	" " "	Intracerebral	Died — 24 hours
12	" "	" " "	"	Died — 12 hours
12	" "	" " "	"	Killed — 62 days

ease. Histological examination of the tissues at the site of injection revealed no significant alterations.

A detailed comparison of these intranuclear inclusions in moles with our own preparations of inclusions in guinea pigs, with speci-

mens showing inclusions in human salivary glands and in rats sent to Dr. E. V. Cowdry by Dr. S. B. Wolbach and by Dr. Juanita Thompson respectively, together with a close study of the literature, brings to light a number of interesting features.

1. The inclusion incidence of 100 per cent of 14 in moles is to be compared with 12 per cent of 183 in humans (Farber and Wolbach [4]), 84 per cent of 75 in guinea pigs (Cole and Kuttner[1]), and 14 per cent of 70 in rats (Thompson [5]).

2. It is not feasible to compare the number of inclusion-containing cells per unit volume of tissue because the observations on species other than the mole are not quantitative. Probably they are equally numerous in the mole, as in the others.

3. The location of the inclusions in moles is in the serous and mucous gland cells and not in the duct cells, whereas in the others the duct cells are often more frequently affected than the gland cells.

4. In the mole, guinea pig and rat the occurrence of inclusions is apparently limited to the salivary glands, but in humans intranuclear inclusions have been found in other tissues of the same individuals (see Farber and Wolbach [4] and their review of literature).

5. The size of the hypertrophied cells is greatest in humans, about the same in guinea pigs and rats and least in the moles.

6. The morphology of the inclusions is similar in all four. Their outlines are irregular, often their central parts are denser. From the periphery strands of material may extend toward the nuclear membrane. The inclusions are not evenly rounded, spherical droplets of material, nor are they hyaline in appearance like Cowdry's type B inclusions.[6]

7. The inclusions are apparently more basophilic in the moles than in any of the others. They also yield a more marked Feulgen reaction for thymonucleic acid and Bensley-Macallum test for masked iron than Cowdry [7] secured with the guinea pig inclusions. Data on thymonucleic acid and masked iron are not available for the human and rat inclusions.

8. Margination of basophilic chromatin on the nuclear membrane is absent or slight in moles — a circumstance that may be related to the retention of basophilia by the inclusions. This margination is very conspicuous in guinea pigs and comparatively slight in humans and rats.

9. The nucleoli behave in the same way in all four species in so far that they do not contribute noticeably toward the formation of the inclusions.

10. A clear, unstaining halo between the inclusion and the nuclear membrane is most noticeable in the guinea pig, less so in the mole, and least, but to about the same degree, in humans and rats.

11. Similar basophilic bodies occur in the cytoplasm of the inclusion-laden cells in all four species. In the mole, however, they are found on all sides of the nucleus and apparently do not show quite the same tendency to be clumped in the distal cytoplasm, between the nucleus and lumen, as in rats, guinea pigs and humans.

12. No constant accompanying reaction or degeneration of the tissue about the affected cells is found in any of the forms, but, in the case of all of them, mention is made of occasional infiltration by lymphocytes and phagocytic cells.

13. No parasites like those herein reported in the oral epithelium and salivary glands of moles, or of any sort, have been described in humans, guinea pigs or rats.

14. Detection of the inclusions in these four species has been to some extent a matter of chance. In none of them is attention drawn to the inclusions by the exhibition of definite clinical symptoms.

Discussion

The intranuclear inclusions in the salivary glands of moles are sufficiently like those in humans, guinea pigs and rats to suggest a more or less common origin. Only in the guinea pig has a virus been identified as the etiological factor, but the opportunity to demonstrate virus action in the others has not been favorable. It is likely that systematic study of the salivary glands in many species will bring to light further instances of the occurrence of similar inclusions in the absence of distinctive signs of disease. The fact that the intranuclear inclusions in this location are so very large does not of itself indicate that the causative agents if, as in guinea pigs, they turn out to be viruses, are peculiar or very different from those that influence other tissues. When the submaxillary virus of guinea pigs is led to act on the brain it produces inclusions that are not particularly large in cells that are not hypertrophied. Consequently it may be supposed that at least two factors are involved — the nature of the virus and the reactivity of the particular cells.

SUMMARY

All of 14 moles examined show, in the absence of clinical symptoms, intranuclear inclusions in their salivary glands which resemble in many respects those previously reported in humans, guinea pigs and rats.

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DESCRIPTION OF PLATE

PLATE 144

FIGS. 1 to 4 inclusive. Drawings of affected cells to show detailed structure of intranuclear inclusions and position of cytoplasmic inclusions. Note the paired inclusion in Fig. 2 and the presence of the nucleolus in Fig. 3. × 2300.

FIG. 5. Drawing of mucous acinus to show relative size and position of the affected cell. Note the encroachment upon the lumen. × 2300.

FIG. 6. Drawing of mucous and serous acini showing involved cells in which cytoplasmic inclusions are absent. × 1300.

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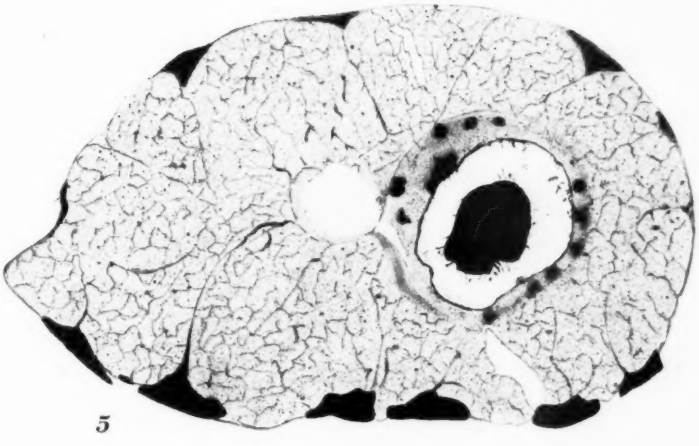

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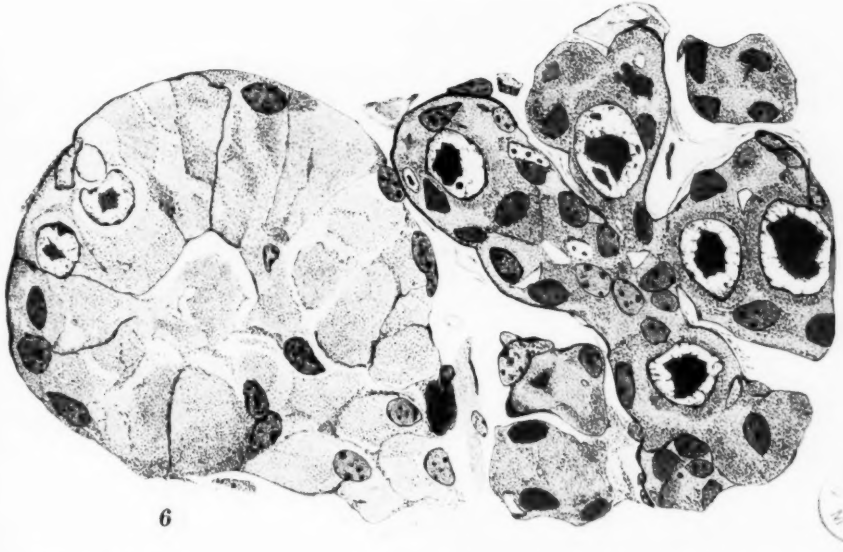

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ENCEPHALOMYELITIS, PROBABLY DUE TO LEAD POISONING *

REPORT OF A CASE

A. B. BAKER, M.D.

(From the Department of Pathology, University of Minnesota, Minneapolis, Minn.)

Numerous cases of accidental lead encephalitis in man, as well as experimental lead intoxication in animals, have been reported in the literature. Kato [1] in 1932 published a complete review of the Japanese literature on this subject and added many new cases of his own. But, in spite of the extensive investigations with animals and the careful study of human material, an agreement as to the pathological lesions in this type of cerebral involvement is still lacking. Friedländer [2] found no alterations in the brain in his case, while Cadwalader,[3] Lehmann and co-workers,[4] and McCarthy [5] reported striking changes in the nerve cells in the brains of both humans and animals dead of lead poisoning.

Lehmann and his co-workers experimented with cats and caused a "liquefaction" of the nerve cells with a destruction of both nucleus and cell body. McCarthy produced cerebral lesions in dogs with lead acetate and noted an extensive chromatolysis and vacuolization of the nerve cells with a slight glial proliferation. Somewhat similar findings were reported by Cadwalader in human material. Hassin,[6] however, in 3 cases found the nerve cells intact. He noted only thickening of the pia arachnoid and slight glial proliferation. Barron and Habein [7] reported a case of lead encephalitis in which the brain was histologically normal. McKhann and Vogt [8] in a recent publication suggested the possibility that much of the cerebral symptomatology in lead encephalitis might be due to an increased intracranial pressure and not to an actual destruction of nerve cells.

Spinal cord lesions from lead poisoning seem to be even less frequent than the cerebral involvement. Bechtold,[9] Sons,[10], Lewin and Treu [11] and Propper [12] have published complete clinical reports of

* Received for publication March 22, 1934.

lead myelitis with recovery, while Spiller [13] and Cadwalader have described the histological changes in the spinal cords of their cases. Spiller found an extensive degeneration of the anterior horn cells with no alteration in the white matter. In Cadwalader's case the pathological findings closely resembled those of amyotrophic lateral sclerosis. The anterior horn cells were severely injured and there was a mild degeneration of the lateral columns. Only a few fibers were destroyed in the posterior columns and these were noted only in the lumbar segments.

In view of the absence of detailed pathological reports dealing with severe lead encephalomyelitis a full report of the following case seems warranted.

Report of Case

Clinical History: J.X. J., a male aged 53 years, was first admitted to the hospital Sept. 22, 1930, complaining of increasing weakness and loss of weight. In January, 1930, he had secured employment, which consisted of dipping metal parts into a tank of lead paint. While working, his forearms and arms were continuously immersed in the paint, which also splashed freely over his neck and face. He felt well until the first part of June, when he began to notice a taste of paint in his mouth and had periodic attacks of nausea and vomiting without any associated abdominal cramps. Early in July an intense skin irritation of the upper extremities developed which was diagnosed as "paint dermatitis" and proved extremely resistant to local treatment. He continued working until August 15th, although gradually losing weight and strength. At the time of his first admission to the hospital he had lost 14 pounds in weight.

The past history was essentially negative. While working in a boiler factory in 1917 his hearing had been impaired. There had never been any gastrointestinal disturbances and, until the onset of his present illness, the patient's weight had remained constant, at about 150 pounds. His father died at the age of 62 years from a carcinoma of the stomach. His mother died at 23 years of age from tuberculosis. He had been married 29 years and his wife is living and in fairly good health.

Physical Examination: The patient appeared anemic; he was quite emaciated and had a sallow, yellowish complexion. There was a grayish discoloration of the hands and forearms with many excoriated lesions scattered over these areas. The pupils were equal and regular and reacted normally to light and accommodation. The ocular movements were unimpaired. There was a marked gingivitis and extensive dental caries but no definite lead line. The tongue showed no atrophy. The lungs were normal. The heart was normal in size, shape and position; rate 76, no murmurs, tones normal. The blood pressure was 142/86. Abdominal examination was negative.

Neurological Examination: The cranial nerves were normal except for a partial nerve deafness in both ears. The abdominal reflexes were decreased but all the deep reflexes were fairly normal Vibration sense was diminished in the ankles and legs, being almost completely absent on the right side. There was no

sign of ankle or wrist drop or of any other muscular weakness. At this examination the neurologist concluded that the findings were insufficient to warrant a positive diagnosis of any organic disturbance of the central nervous system.

The patient remained in the hospital for 19 days and, after his discharge, reported to the outpatient department for further observation and study. Throughout this period numerous laboratory studies were performed. The urinalysis was negative on four occasions. The icterus index was 12 units. Gastric expression revealed no free hydrochloric or lactic acid but contained 10 degrees of total acidity at 10 minutes, and 20 degrees at 20 minutes. The stools contained no pus, mucus or occult blood, and microscopic examination was negative for ova and parasites. The Wassermann test was repeatedly negative, as was also the spinal fluid examination. Roentgen-ray studies of the chest and abdomen gave normal findings. The blood studies are tabulated in Table I.

TABLE I

Examinations of the Blood

Date	Red cell count	Hemoglobin	Color Index	White cell count	Reticulocytes
		per cent			*per cent*
9/19/30	2,670,000	82	1.6	6750	
9/21/30	2,840,000	79	1.4	7300	
9/29/30	2,000,000	70	1.7	7000	20.0
10/ 1/30	2,630,000	66	1.2		
10/ 7/30	2,520,000	67	1.3	4850	1.0
10/ 9/30	2,340,000	65	1.4	6600	1.8
10/10/30	2,260,000	62	1.4	6400	1.3
10/11/30	2,010,000	60	1.5	6150	2.0
10/14/30	2,440,000	61	1.3	6650	1.4
10/18/30	2,150,000	60	1.4	8050	2.0
10/22/30	2,130,000	57	1.3	6300	2.8
10/24/30	2,470,000	47	0.95	6850	7.5
10/29/30	2,040,000	59	1.6	7600	3.3

The blood smears were studied repeatedly by the department of hematology and were described as follows. "The red cells show the following features: anisocytosis (macrocytes, normocytes and microcytes), poikilocytosis, hyperchromasia, occasional polychromatophilic cells and occasional stippled cells (the basophilic stippling was increased after administration of potassium iodide). The neutrophil leukocytes are present in normal numbers but are toxic, their nuclei showing a marked 'shift to the right.' These cells were given careful study because the neutrophils of pernicious anemia usually show a similar change, but these cells are not of the pernicious anemia type, since their nuclear lobes are too plump and their neutrophil granules are not of the right type. It may be concluded that the patient's anemia is of a toxic type and compatible with lead poisoning but not characteristic of pernicious anemia."

After the patient's discharge from the hospital his symptoms gradually became augmented. An attempt was made to de-lead him with potassium iodide, but this medication caused a definite exacerbation of symptoms, a marked increase in the basophilic stippling of the red cells and a drop in the hemoglobin of

13 per cent in a period of 6 days (60 per cent to 47 per cent). A milk diet was then prescribed and calcium lactate given in large doses by mouth. On this type of therapy, which tends to keep the lead stored in the bones, he promptly improved and the hemoglobin increased 12 per cent in the next few days. This experience in treatment seems to us to be of definite diagnostic significance.

Throughout his illness the hemoglobin remained at about 50 per cent and resisted all attempts at correction. Liver extract was given orally over a long period with no results, although three injections of liver intramuscularly were followed by a temporary rise in the hemoglobin, the red cells remaining low.

By August, 1932, nearly 2 years after the onset of the ailment, weakness had become so marked that it greatly interfered with walking. A definite spasticity of the lower extremities had also developed. The weakness and spasticity advanced rapidly, so that by January, 1933, the patient could no longer walk alone and was confined to bed, where he remained until death. A neurological examination was performed in November, 1932, at which time the patient was so weak that he was unable to walk alone. All voluntary movements of the arms and legs could be executed, although he was manifestly ataxic. None of the deep reflexes was elicited even with reinforcement. The position and vibration senses were entirely absent in all extremities, while the deep tendon and muscle sensations appeared only slightly impaired. Superficial sensibility was intact. The findings at this examination seemed compatible with a subacute combined degeneration of the cord.

During the last 6 to 8 months of life definite signs of mental involvement developed. He would become disoriented and talk irrationally for hours. Terminally a urinary retention developed. He died on June 27, 1933, after having been bedridden for 6 months.

Postmortem Examination

The body was that of a poorly nourished, elderly male, 161 cm. long, weighing about 110 pounds. There appeared to be moderate atrophy of the muscles of the upper extremities but this may have been due to the extreme degree of undernourishment. The abdominal muscles were well developed. A large decubitus was present over the sacrum.

The peritoneal cavity showed nothing of note. The right pleural cavity, anteriorly and inferiorly, was obliterated by dense fibrous adhesions; the left pleural cavity as well as the pericardial sac was normal.

The heart weighed 200 gm. and was normal. The coronaries were sclerotic and their lumens were very much narrowed in places, although at no point completely closed. Each lung weighed 300 gm. and contained a moderate amount of edema and congestion but no free pus. The spleen weighed 200 gm. and showed no disease.

The liver weighed 1300 gm. Its surface was smooth. On section

the interlobular spaces were more conspicuous than normal and there was a slight yellowish tinge to the parenchyma. The gall-bladder and ducts were all normal The gastro-intestinal tract, pancreas, adrenals and kidneys showed no disease.

Microscopic Examination

Microscopic examination of the organs confirms the gross impressions. The liver shows a moderate fatty metamorphosis and, with special stains, no hemosiderin can be detected in the liver tissue.

The brain and spinal cord were carefully removed and immediately fixed for special staining. In gross the brain showed nothing of note but the entire cord was unusually small. The dura mater and pia arachnoid appeared normal, not showing the thickening often mentioned by other investigators.

Sections were taken from several cortical areas, the basal ganglia, midbrain, cerebellum, medulla oblongata and various levels of the spinal cord. These were stained in the following ways: hematoyxlin-eosin, Weigert's myelin sheath stain, iron hematoyxlin-Van Gieson, Nissel's method, Bielschowsky's stain, and sudan III.

Throughout the cerebral cortex there is a marked shrinking of the ganglion cells, leaving a large clear space about the remains of each. In certain of these cells the dendrites are intact, in some scarce or absent, while in others they are fragmented and separated from the cell body. In many cells the cytoplasm is almost entirely absent, leaving only a nucleus situated in a large clear area; in others the nucleus is surrounded by an irregular border of cytoplasmic substance (Fig. 1). The nuclei of the cortical ganglion cells are usually intact and occupy a central or exceptionally a peripheral position. Occasionally the nuclei are shrunken, deeply staining, and separated from the cytoplasm by a zone of varying width (Fig. 1). Nucleoli are usually present. In some areas of the brain, chiefly the temporal lobe and the basal nuclei, another type of change is noted. Here the ganglion cells are swollen to almost twice normal size; their cytoplasm is homogeneous and glossy and the processes are usually absent, giving the cells a rounded edematous appearance (Fig. 2). The nuclei of these large cells are still intact but many have irregular edges and stain poorly. Careful study of the sections reveals all stages from the

slightly swollen cells to those exhibiting complete homogeneity and disintegration. With the special Nissl stain it is readily apparent that many cells have almost completely lost the Nissl substance (Fig. 2). There is only slight indication of neuronophagia in spite of the partial destruction of the nerve cells. Satellites are absent.

Sections from the floor of the fourth ventricle reveal many small hemorrhages, chiefly of a perivascular distribution. The brain tissue around these hemorrhages does not seem to be injured. There is no cellular infiltration or injury to the white matter.

In contrast to the brain the most constant damage to the spinal cord is in the white substance, the gray matter, as a rule, remaining free from injury.

The cervical cord presents the most marked changes. With the Weigert stain a complete demyelinization is noted in the columns of Goll and Burdach, with a partial involvement of the dorsal and ventral spinocerebellar tracts, and of the lateral and ventral corticospinal tracts (crossed and direct pyramidal) (Fig. 3). The direct pyramidal tract presents an asymmetrical involvement showing a more extensive demyelinization on the right side of the cord where the destruction extends into Marie's column (fasciculus sulco-marginalis). With the hematoxylin-eosin and iron hematoxylin stains many cells are detected invading the degenerated white matter. These cells contain a nucleus which is polymorphic and deep staining and a cell body that either is quite without definition or is fairly large, granular and irregular. A fat stain proves these cells to be macrophages in the various stages of development.

The gray matter presents but few alterations. A few of the anterior horn cells are shrunken, although none show any chromatolysis and there does not appear to be a decrease in their number. Numerous dilated blood vessels are scattered throughout the gray substance but no hemorrhages, either diffuse or perivascular, can be detected. The anterior and posterior rootlets are normal, showing no demyelinization and no involvement of their axis cylinders. There are no signs of any inflammation (Fig. 3).

In the midthoracic cord the changes are almost the same as in the cervical region except that the demyelinization is less in the medial parts of the posterior columns. There is still an involvement of the medial and ventral parts of the anterior columns of white matter, in spite of the fact that, at this level, the direct pyramidal tract usually

has decussated and therefore is absent. The anterior and posterior horn cells appear normal, both in number and in structure. In the nucleus dorsalis (Clark's column) the cells are greatly decreased. The few that are present have no processes and appear rounded, with smooth edges, and are diminished in size. Their nuclei are, as a rule, absent. The rootlets are unaffected and the arteries are normal.

The lumbar cord presents the least involvement (Fig. 4). There is only a partial destruction of the posterior columns and the crossed pyramidal tracts. The marked macrophage reaction is still seen in the demyelinated areas. The anterior horn cells and the rootlets are unaffected.

The appearance of the cord with the fat stain is similar in all the destroyed areas. All stages of transformation in the macrophages can be observed, from cells that contain only a few fat granules to others that are completely filled. These cells vary in appearance from small uniform ones with a central nucleus to large irregular cells in which the nucleus is either forced to one side or entirely invisible because of the large fat droplets. Often the macrophages clump together, presenting the appearance of a large mass of fat globules with an occasional small dark nucleus visible in the fatty mass. There is a great tendency for these cells to migrate to the blood vessels, all the vessels in the demyelinated areas being surrounded by collars of fat-filled cells. In many of the vessels the macrophages must have penetrated the walls, the lumens being completely filled with fat.

Discussion

The pathological study clearly indicates that the lesions in our case are almost entirely degenerative in nature and differ greatly in the various parts of the central nervous system. In the brain the ganglion cells of the gray substance are involved, while in the spinal cord most of the damage is in the white substance, giving the appearance of a combined degeneration. Such a degenerative process is usually considered as a primary alteration, due to the selective toxic influence of the lead on the various parts of the nervous system, but the reason for the great difference in localization of the destructive process in the central nervous system is at present unknown.

SUMMARY

1. A case of encephalomyelitis is reported in a male who was thoroughly exposed to lead paint for a period of 7 months.

2. A pathological study of the nervous system revealed a marked destruction of the nerve cells in the cerebral cortex and an extensive demyelinization of various tracts in the spinal cord.

3. The literature dealing with lead encephalomyelitis is reviewed.

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DESCRIPTION OF PLATES

PLATE 145

FIG. 1. Section through the cerebral cortex showing the marked shrinking and irregularity of the nerve cells.

FIG. 2. Drawing showing some of the ganglion cells of the temporal cortex. All the cells are swollen and present a marked chromatolysis. The cell marked "a" is an axone. In cells "b" and "c" there is also a destruction of the processes, giving the cells a rounded appearance.

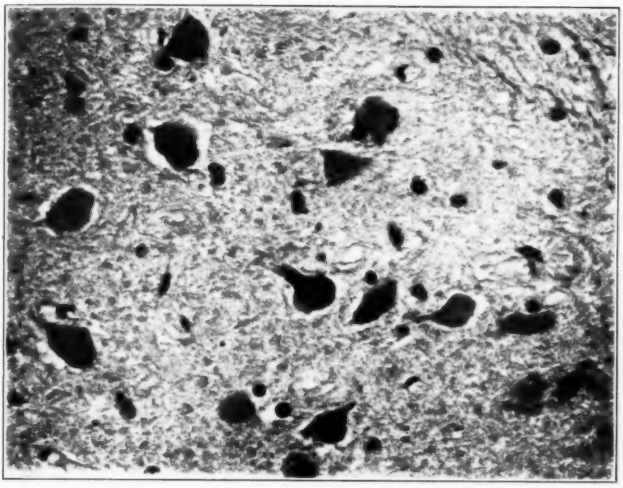

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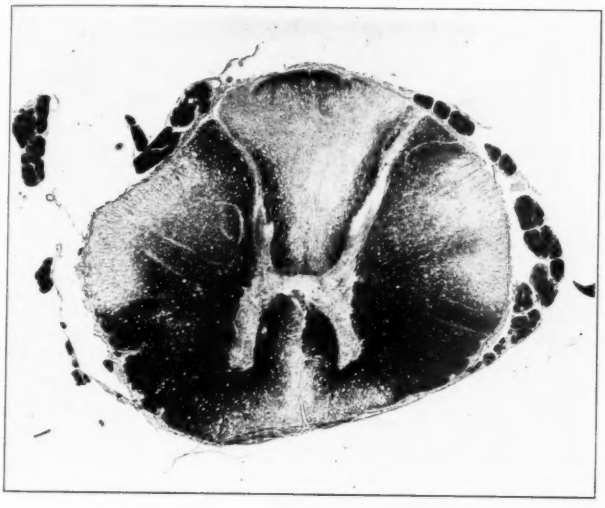
PLATE 146

FIG. 3. Section through the cervical cord showing the striking demyelinization of the posterior, the lateral, and part of the anterior columns. The rootlets are intact.

FIG. 4. Section through the lumbar cord. There is only a partial destruction of the posterior columns and the crossed pyramidal tracts.



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4

UNIDENTIFIED PARASITE IN HEART MUSCLE *

WILLIAM C. VONGLAHN, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City)

In the routine study of heart muscle from a case of aortic stenosis and insufficiency peculiar bodies were discovered in the myocardium. Since a thorough search of the literature failed to reveal any record of similar bodies having been described in the heart it seemed advisable to report the case.

The patient (History No. 316,149, Autopsy No. 10,821), a carpenter, aged 63 years, born in Virginia, was first admitted to the Medical Service of the Presbyterian Hospital because of cardiac decompensation. He was found to have aortic stenosis and insufficiency. At the end of 4 weeks the cardiac condition was sufficiently improved to permit him to return to his home. That evening he had a chill and came back to the hospital. It was determined that he had lobar pneumonia due to pneumococcus type II. He died on the 4th day of this illness.

At autopsy the heart was found to be hypertrophied, weighing 500 gm. The aortic valve was extensively calcified. The middle and lower lobes of the right lung were consolidated.

Peculiar solid bodies were found lying within the sarcoplasm of the hypertrophied heart muscle. The end of the body adjacent to the nucleus was bluntly rounded, the other end pointed. Near the bluntly rounded end was an oval vesicular nucleus containing one or more chromatin particles, and close to this nucleus, in some instances, a solid round structure. One or more oval vacuoles were present, often near the pointed end. The bodies averaged 52.5 microns in length and 5.5 microns in width. They were usually straight, except for slight undulation. In Figure 1 is shown a photographic reconstruction of one of these structures. One body was found that was turned abruptly at right angles close to the muscle nucleus, another was sharply bent upon itself.

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Another of these bodies was divided longitudinally through part of its length, so that it was roughly Y-shaped. There were two rounded ends directed toward the nucleus; the other end was sharply pointed. In one of the limbs with the rounded ends were two nuclei, in the other, a single nucleus. A large vacuole was present just where the two limbs joined.

Two larger bodies were discovered that had been cut across in sectioning the block of muscle. One of these fragments measured 63 microns in length and 7 microns in width; near the rounded end was a huge nucleus 5.8 microns wide in which were three nucleoli, the largest 3.8 microns in diameter. The nucleus was constricted at its midportion (Figs. 2 and 3).

The fragment of the second body likewise had a rounded end; it measured 45.5 microns in length and 7 microns in width. The oval nucleus was 10.5 by 4.5 microns. The single nucleolus was 2.4 microns in diameter (Fig. 4).

In many of the muscle fibers fragments were found and it was obvious that they were parts of more than one of these bodies.

The staining reactions were as follows.

Stain	Bodies
Hematoxylin-eosin	Eosinophilic
Gram	Gram-positive
Mallory's phosphotungstic acid hematoxylin	Yellow
Heidenhain's iron hematoxylin	Black
Carbol fuchsin followed by 1% acid alcohol	Not acid-fast
Giemsa	Nucleus deep blue, remainder robin's egg blue
Eosin-methylene blue, azur B	Nucleus very deep blue, remainder dark blue

The only internal structure that could be distinguished in these bodies, aside from vacuoles and nucleus with its nucleolus and chromatin material, were fine granules in the bluntly rounded portion. In the phosphotungstic acid hematoyxlin preparations the peripheries of the bodies were refractive.

These bodies were not encapsulated; they seemed somewhat rigid in the fixed preparation. They appeared to be surrounded by clear fluid. They were situated always adjacent to the muscle nucleus with the long axis parallel to the direction of the myofibrils. The nucleus of the muscle was always indented or invaginated by the

bodies. The myofibrils were pushed to either side by the bodies, but were otherwise unchanged.

The heart muscle containing these bodies was not enlarged, as compared with adjacent muscle that had none of them. There was no inflammatory reaction about muscle containing the bodies.

The bodies were very numerous in the posterior part of the left ventricle and were less frequently observed in the muscle from the apex of this ventricle. None could be found elsewhere in the heart. The only voluntary muscle available for histological study was the diaphragm; none of these bodies could be discovered in it.

These bodies do not conform to any known form of degeneration. Their definite shape and well preserved nuclei, together with their staining reactions, would indicate that they are not simple degeneration products. They do not resemble any hitherto described parasite known to lodge in heart or voluntary muscle.

Preparations have been shown to many pathologists and protozoölogists. The consensus of their opinion was that these bodies were of parasitic nature but none could identify them. It is not believed that the bodies had any part in the production of the hypertrophy of the heart.

DESCRIPTION OF PLATES

PLATE 147

FIG. 1. Photographic reconstruction of a body within a single muscle fiber. Due to slight undulation the entire body could not be brought into focus; it was photographed in segments and these matched as accurately as possible. Gram's stain. × 2050.

M, N, indented heart muscle nucleus.

N, nucleus of body.

V, vacuoles in body.

II

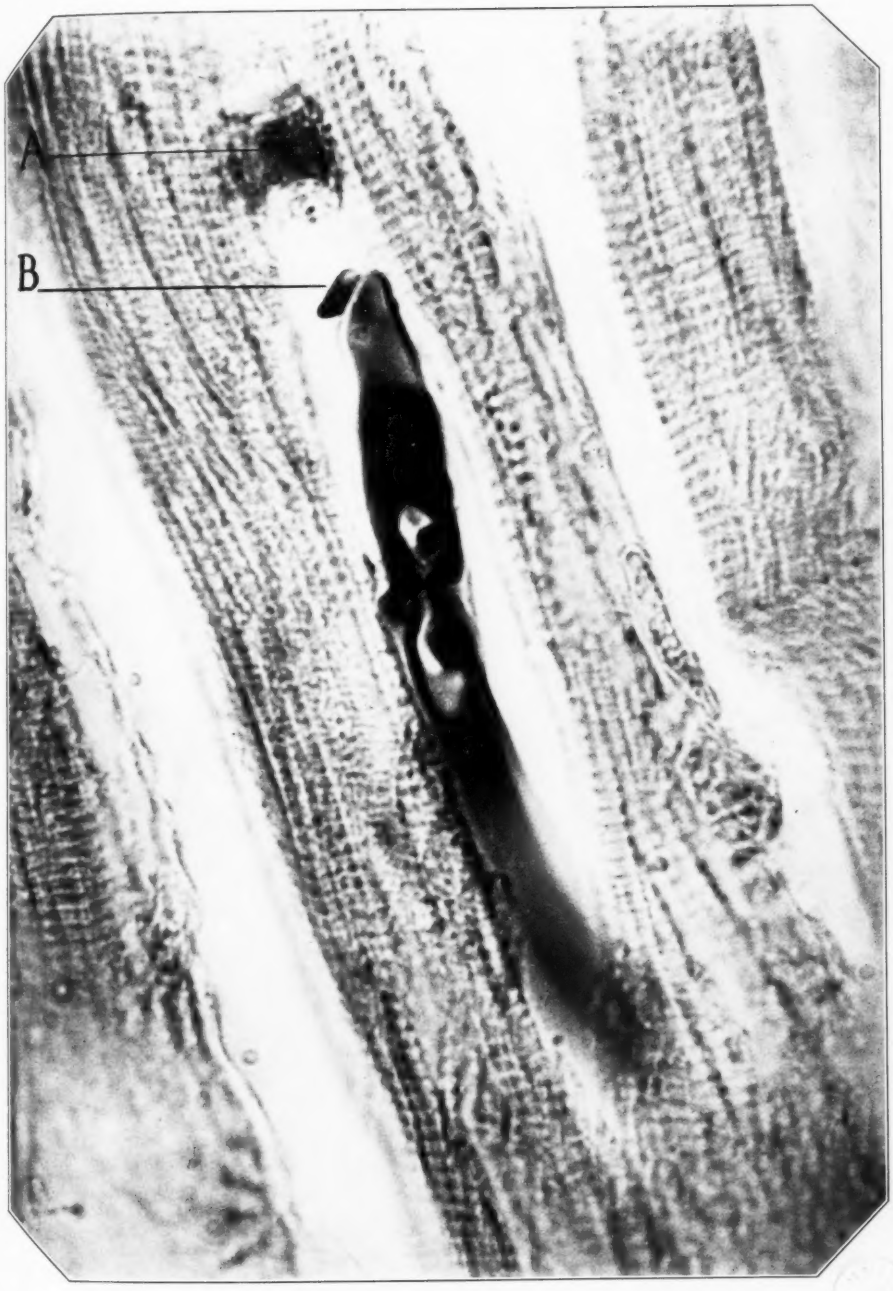
Unidentified Parasite in Heart Muscle

PLATE 148

FIG. 2. Fragment of a body photographed at slightly different levels. The constriction of the nucleus and two nucleoli is shown. Gram's stain. × 2100.

A. indented heart muscle nucleus.

B. red blood cell that has floated into the space about the body.



2

VonGlahn

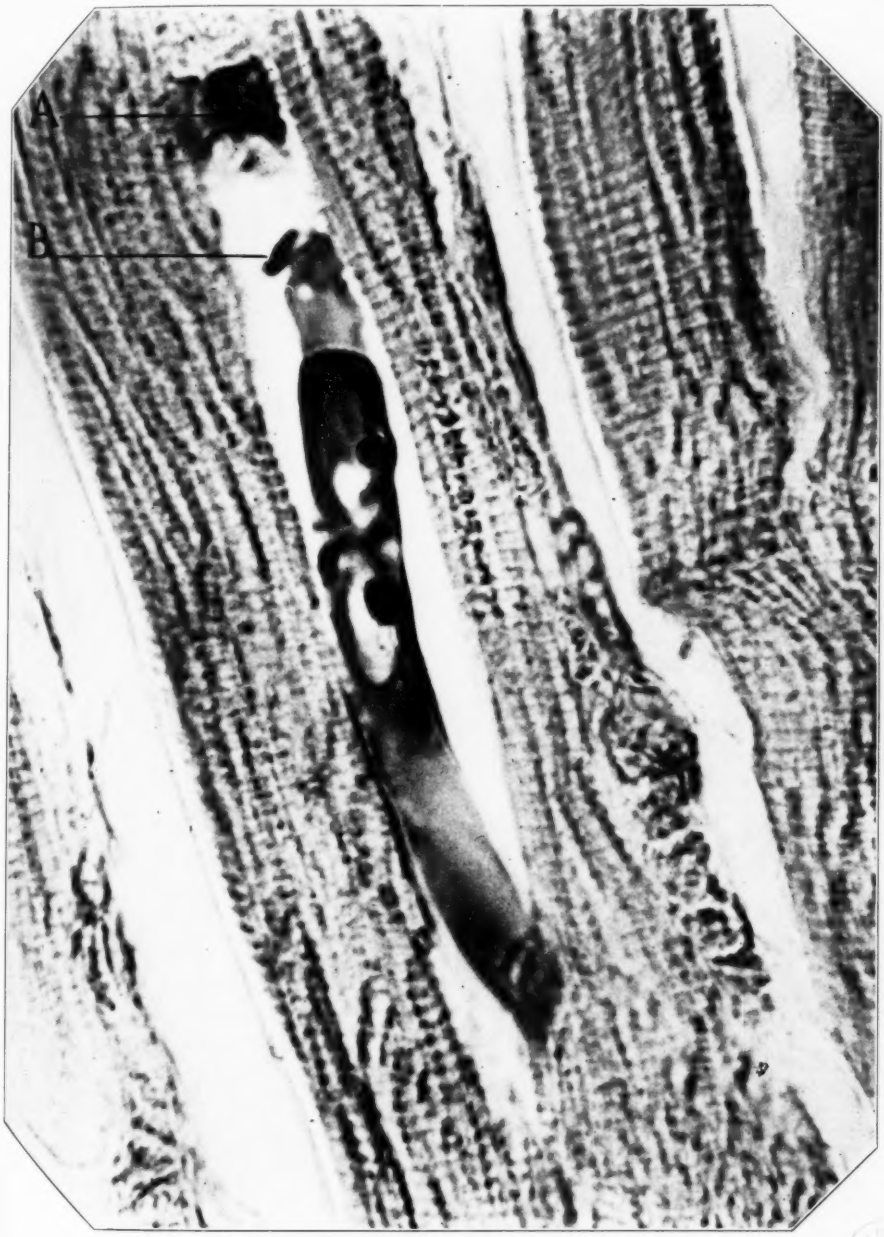
Unidentified Parasite in Heart Muscle

PLATE 149

FIG. 3. Fragments of a body photographed at slightly different levels. The third nucleolus is shown in this figure. Gram's stain. × 2100.

A. indented heart muscle nucleus.

B. red blood cell that has floated into the space about the body.



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VonGlahn

Unidentified Parasite in Heart Muscle

PLATE 150

FIG. 4. Fragment of a body. Gram's stain. × 2100.

4

VonGlahn

Unidentified Parasite in Heart Muscle

TUBERCULOSIS OF THE MAJOR BRONCHI *

HERBERT S. REICHLE, M.D., AND THOMAS T. FROST, M.D.

(From the Department of Pathology of the Cleveland City Hospital and Western Reserve University, Cleveland, Ohio.)

Little attention has been given in the past to tuberculosis of the major bronchi.[1] Ranke's discussion, though valuable, is cursory and his remarks are scattered among his studies on the classification of tuberculous disease.[2] Tuberculosis of the major bronchi is, however, of more than descriptive interest since it not infrequently leads to bronchiectasis; the rigidity and size of the diseased bronchi may impede collapse of the lung and interfere with the natural healing of cavities. Furthermore, the studies herein described reveal that the pathogenesis of tuberculosis of the major bronchi has unique features not found in other organs.

In this article the term "major bronchi" refers to air passages that possess mucosa, submucosa, muscle, elastica, mucous glands, an encircling wall of cartilage and a definite adventitia. The material for study was obtained from 37 routine unselected cases of pulmonary tuberculosis, mostly chronic phthisis (tertiary isolated pulmonary tuberculosis of Ranke's classification), but also cases of childhood tuberculosis (primary pulmonary tuberculosis with generalization) and of miliary tuberculosis. The lungs were either fixed by injection of formalin into the trachea after removal from the chest, or hardened *in situ* by injection of formalin into the inferior vena cava. Identified blocks were cut, embedded in paraffin in the usual fashion and stained with hematoxylin-eosin.

Gross examination of the bronchi yielded little of importance. No necrosis of the major bronchi was found although a thickening of the mucosa was often present. The gross appearance was deceptive, since an apparently well preserved wall might be the seat of extensive tuberculosis. Diffuse involvement which apparently would preclude a restoration *ad integrum* was found, however, only in phthisis with extensive cavitation or gross bronchiogenic spread, and even here bronchial mucosa which was but a few centimeters removed from the active focus was usually free from histologically specific

* Received for publication April 27, 1934.

signs of tuberculosis. In such cases edema and a slight diffuse infiltration with lymphocytes were the only evidences of disease, an observation that is particularly striking since large quantities of infectious sputum must have passed over this mucosa daily for weeks and perhaps months. In this fact is found one of the principal arguments against the assumption that tuberculosis of the bronchi usually develops by implantation, a hypothesis that has hitherto seemed plausible to some observers because of a specious comparison of bronchus with intestine. Intestine, however, has an absorbing surface whereas the bronchus has not; in fact, the latter is equipped with strong barriers against the invasion of microörganisms by the possession of cilia, mucous glands and peristaltic activity.

The most striking characteristic of tuberculosis of the major bronchi is the tendency for the disease to affect the mucous glands. The earliest manifestations of tuberculous adenitis cannot be distinguished from the simple lymphatic infiltration noted above. Tubercles appear which may be of the dense proliferative type or may consist of a central core of caseation with a wide zone of perifocal reaction. In either case the process is definitely *between* the acini of the gland. The latter are pressed aside, either undergoing atrophy or, owing to obstruction of their ducts, becoming dilated. This is additional evidence that the infection does not proceed from the bronchial lumen by way of the collecting tubules of the mucous glands.

The predilection of tuberculosis for the mucous glands is all the more striking because glands of the same structure and analogous functions elsewhere, *e. g.* salivary glands and pancreas, are not especially prone to show tuberculous disease. Furthermore, the mucous glands themselves have rarely shown any disease in cases of pure acute miliary tuberculosis, although here and there a few scattered tubercles may be found in the foci of lymphatic cells of the submucosa. Tuberculosis of the mucous glands is therefore usually associated with a tuberculous bronchitis and is probably not due to a hematogenous distribution.

An explanation for the rôle which the mucous glands play in tuberculosis of the major bronchi is found in the minute anatomy of these structures. On the basis of publications by Lewis and Stöhr,[3] Piersol,[4] Macklin,[5] Berry, Brailsford and Daly,[6] and Miller,[7,8] it is possible to outline the pertinent data. Cartilage, muscle and elastic

tissue of a major bronchus represent an almost faultless wall interposed between submucosa and a rather loose adventitia. The main groups of mucous glands are found between the cartilage and the muscle. External to the cartilage lobules of these glands are also found lying in the adventitia, hugging the ring of cartilage on one side and in intimate contact with the peribronchial alveoli and lymph nodes on the other. Ducts from these lobules pass through the elastic membrane between the links of the cartilage, pierce the muscle and internal elastic membranes to enter the submucosa and finally reach the lumen of the bronchus. Sometimes this extracartilaginous glandular tissue is but an outpouching of the mucous glands internal to the ring of cartilage. In the adventitia and often close to the extracartilaginous glands are the lymphatics and venous tracts which transport fluid from the periphery of the lung to the hilum. All the highly infectious material carried from a parenchymal focus must in large part pass through these avenues. The lymphatic flow in the bronchus itself is from the submucosa out toward these main pathways; it is therefore centripetal. Veins and arterioles pass through the same clefts as the extracartilaginous glands.

This portion of the mucous glands is a portal of entry in tuberculous bronchitis and the mode of infection may be called "*infection by contiguity.*" The source may be a tuberculous pneumonia of the peribronchial alveoli. Such a condition is found in and near cavities where the bronchial wall usually resists destruction much longer than the parenchyma, and the tuberculous process outflanks, as it were, the bronchus. More important, however, are tuberculous lymphangitis and phlebitis of the vessels which pass toward the hilum in immediate proximity to the extracartilaginous glands. In extensive disease of the parenchyma such changes are practically never absent and unless fibrotic induration of the bronchial adventitia with atrophy of the extracartilaginous mucous glands occurs, a spread to the interior of the bronchus is inevitable. Diseased lymph nodes furnish another source. Tuberculous disease which affects the peripheral portions of these nodes is prone to spread to the adventitia of the bronchus and from there to the mucous glands. Severe pneumoconiosis will develop exactly the same condition and it has also been described in tumor metastases.[9]

Implantation tuberculosis of the major bronchi appears to occur usually after massive irruptions of caseous material. It may be seen

in secondary bronchi of a lower lobe which is the seat of a bronchiogenic distribution from a large apical cavity. Apparently the mass of the infectious material and the effect of gravity in the bronchi of lower lobes counteract the protective action of cilia, mucus and bronchial peristalsis. Hence implantation tuberculosis was not seen in the larger bronchi draining the upper lobes, even though the blocks were taken but a few centimeters from a large cavity.

In these cases a different mechanism of infection prevails — *infection by continuity*. Tuberculous granulation tissue and caseation creep by degrees along the submucosa, destroying the mucous glands, elastica and muscle even before the mucosa itself disappears. Cartilage resists the destructive influences for a longer time and may be found in the midst of tuberculous granulation tissue. Its ultimate destruction is apparently consummated by a metaplasia into a fibrillar substance which is arranged tangentially to the central cartilage and in which are found small pyknotic nuclei. In some instances granulation tissue erodes the cartilage and bone formation occurs. In infection by continuity the mucous glands are not selectively chosen for attack; the pathological process infiltrates all tissues. That even under these disadvantageous circumstances the bronchial wall exhibits considerable resistance was shown in several instances by sections of serial blocks cut from main bronchi draining large tertiary apical cavities. The wall of the primary bronchus was well preserved; here and there a few tubercles were found in mucous glands or submucosa, but for the most part nothing but edema and a sparse infiltration with lymphocytes was seen up to the point where the secondary bronchi entered. Here the wall of the main bronchus showed a rather abrupt atrophy; muscle and elastica disappeared, the cartilage dwindled in size and the submucosa became a mass of granulation tissue in which were found islands of atrophic mucous glands. This granulation tissue led directly into the pyogenic membrane of the cavity and it was not possible to determine where the secondary bronchi ended and the cavity began. Even far in advance of the tuberculous process fibrosis of the adventitia and atrophy of the extracartilaginous mucous glands were apparent; thus the bronchus is protected from the effects of contiguous disease. Collapse is, however, impeded and obliteration of the lumen prevented by the dense hyalinized connective tissue which forms in adventia and submucosa.

Summary and Conclusions

Tuberculosis of the major bronchi can be classified according to pathogenesis as (1) infection by implantation, (2) infection by contiguity, and (3) infection by continuity. The first is decidedly less common than the other two, a fact that may be ascribed to the protective influences of cilia, mucus and bronchial peristalsis. Infection by contiguity is prone to occur because of the proximity of the extracartilaginous mucous glands to diseased lymphatics and lymph nodes. Proliferation of the adventitia and atrophy of the mucous glands tend to close this avenue. Infection by continuity occurs secondarily to an implantation tuberculosis in the lower lobes and in the bronchi draining a tuberculous cavity. Major bronchi appear to be especially resistant to tuberculosis; however, when fibrosis has transformed them into rigid tubes and mucosa and submucosa are destroyed and replaced by tuberculous granulation tissue, an open focus of disease is created which is rarely closed either by natural processes of healing or by pneumothorax or thoracoplasty.[10] We therefore suggest that the critical aspect of a tuberculous cavity is not so much its size *per se* or the density of its wall, as its relation to a major bronchus.

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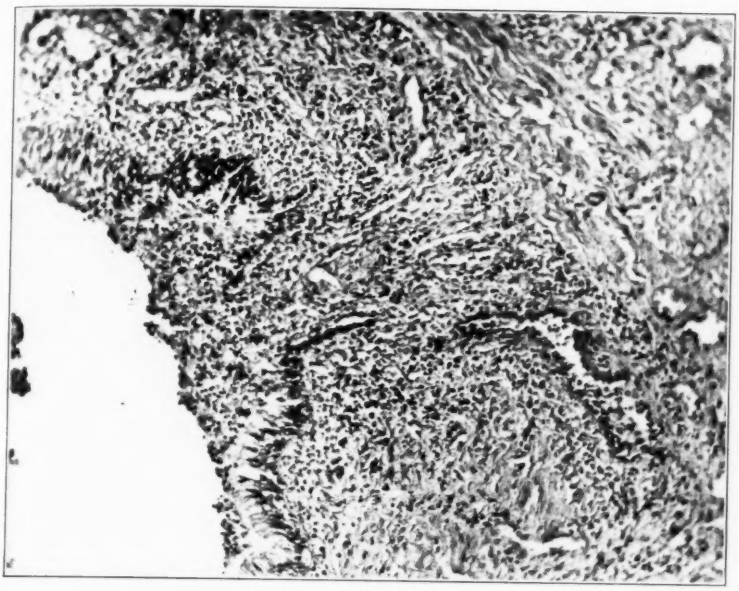
DESCRIPTION OF PLATES

PLATE 151

FIG. 1. Implantation tuberculosis. Note interruption of mucosa by tubercle with development of tuberculous granulation tissue in submucosa. Hematoxylin and eosin stain. × 100.

FIG. 2. Tuberculous adenitis of submucosal mucous gland. Hematoxylin and eosin stain. × 70.



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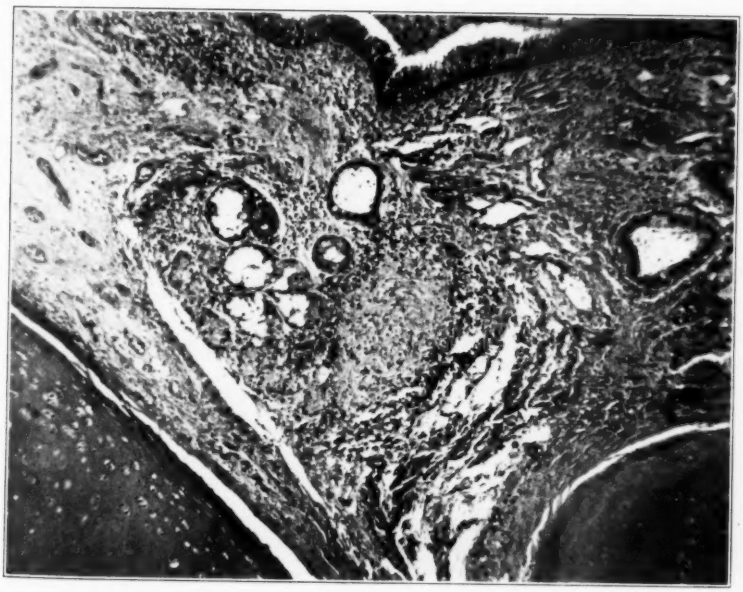

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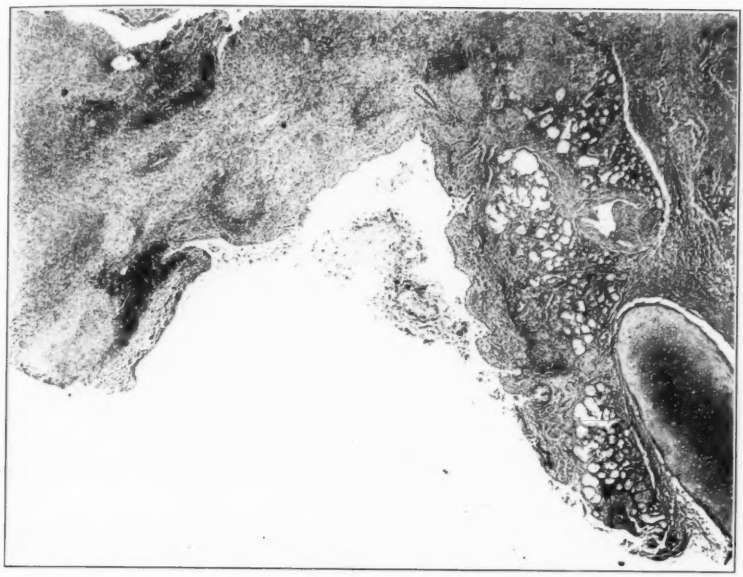
PLATE 152

FIG. 3. Longitudinal section of a bronchus showing advanced tuberculous disease by continuity. Note preservation of mucosa far into diseased area. Hematoxylin and eosin stain. × 10.

FIG. 4. Infection by contiguity. Tuberculous lymphadenitis with invasion of submucosa between clefts of cartilage. Note tuberculous disease of lymph vessels or venules and their proximity to extracartilaginous mucous glands. Hematoxylin and eosin stain. × 10.



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4

UNIV. OF MICH.

SCIENTIFIC PROCEEDINGS OF THE THIRTY-FOURTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

HELD AT THE BANTING INSTITUTE,
UNIVERSITY OF TORONTO,
TORONTO, ONTARIO,
MARCH 29 AND 30, 1934

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

President AVERY in the Chair

The Secretary presented the nomination of the Council for officers as follows:

President	WILLIAM BOYD
Vice-President	N. CHANDLER FOOT
Treasurer	F. B. MALLORY
Secretary	HOWARD T. KARSNER
Incoming Member of Council	E. B. MCKINLEY
Assistant Secretary	ALAN R. MORITZ

Voted unanimously to elect those nominated.

Voted to elect the following new members:

Arthur L. Amolsch
A. B. Baker
Eustace L. Benjamin
Louis F. Bishop, Jr.
Maurice Brodie
Jesse L. Carr
Albert E. Casey
W. H. Chase
Mortimer Cohen
William D. Collier
Martin H. Dawson
Edgar C. Fielden
Leroy D. Fothergill
Olive Gates
Philip H. Greey
Arthur W. Ham
Theodore R. Helmbold
Louis A. Julianelle
Harold B. Kenton
Morton McCutcheon
Herbert S. Reichle
Paul D. Rosahn
Nathan A. Womack
David A. Wood
Angus Wright

It was also voted to reinstate Dr. Harold E. MacMahon.

Voted to accept with regret the resignations of Drs. William Bloom, J. W. Churchman, H. R. Dean, E. C. Dickson, W. W. Ford, W. C. Quinby, O. H. Schultze, J. D. Weis, Benjamin White, Anna W. Williams and L. B. Wilson.

Voted to record with deep regret the deaths of Drs. L. H. Braafladt, F. P. Gorham, A. L. Grover, P. E. McNabb and W. Ophüls.

The Secretary reported that the Symposium for 1935 would be held in collaboration with the American Association of Immunologists on the topic of Virus Diseases, and that Dr. T. M. Rivers had been selected as referee.

The Secretary announced that the next annual meeting of this Association will be held at Cornell University Medical College, New York City, April 18 and 19, 1935.

The Scientific program followed.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Preliminary Observations on the Chemical Relationship of Antibodies to their Antigens.

H. J. Perkin (by invitation), Toronto, Canada.

Abstract. The purpose of this investigation was to study the chemical relationship between the antigen, antibody and the precipitin compound formed by their combination. Iodo-albumin was chosen as a suitable antigen since it produces a strong antiserum and is capable of quantitative analysis in the presence of other proteins. A very pure form of iodo-albumin containing 9.55 per cent of iodine was prepared. It was demonstrated that 40 mg. of this protein injected intravenously into rabbits induced maximum antibody production.

The blood iodine of rabbits immunized against iodo-albumin was found to be increased above the normal and control group. The greater part of this iodine was organically combined and present in the serum, although there was no direct relation between the increased blood iodine and the degree of immunity.

Addition of antigen to immune serum did not show precipitation of all the added antigen. However, the supernatant serum still showed precipitation on further addition of either antigen or antibody. A point of optimum precipitation with relation to the amount of antigen and certain conditions of precipitation was determined. Under these optimum conditions iodine analysis of precipitated and "non-precipitated fractions" showed that less than 50 per cent of the antigen was precipitated. Dissociation of the insoluble precipitate to form a soluble fraction was demonstrated when the two fractions were allowed to remain in contact.

The original purpose of determining whether the increased iodine in the immune serum was associated with the antibody could not be accomplished by the methods used, since the iodine of the serum represented only 5 per cent of the antigen iodine necessary to precipitate it, and the experimental error in iodine estimations of such small amounts was 6 per cent.

Recovery of 3.4 mg. of precipitin with an iodine content of 0.7 per cent suggested that if a chemical union takes place it might be in the proportion of 1 part of antigen to 13 of antibody, assuming that no other serum proteins were carried down in the precipitate. Analysis, after washing the precipitate, suggested that perhaps the antibody may be precipitated by the antigen without the latter necessarily forming any fixed union. However, the experimental evidence to date is inadequate to justify the drawing of any definite conclusions concerning the biochemical relationship between the antibody molecule and the corresponding antigen.

Discussion

(Dr. W. L. Holman, Toronto.) Realizing the large amount of antigen found necessary in producing precipitation with the sera of rabbits after prolonged periods of immunization, we are planning to use sera of animals at an earlier stage in the process when, according to Manwaring, the precipitin complexes

are apparently larger in size but fewer in number. If sufficient precipitate for the iodine determinations can thus be obtained we may approach close to proportions of antigen and antibody, which may help in solving our original problem by the analysis of the possible iodine content of the antibody complex.

THE PREPARATION, PROPERTIES AND APPLICATIONS OF LYOPHILE SERUM PROTEINS AND COMPLEMENT. Stuart Mudd and (by invitation) John Reichel, Earl W. Flosdorf and Harry Eagle, Philadelphia, Pa.

Abstract. Certain applications of familiar conditions, namely the use of low temperature and absence of moisture and oxygen, for preservation of labile substances are here presented.*

A practical method and apparatus is described by means of which water may be removed under a high vacuum (0.005–0.05 mm. Hg.) from biological products in a frozen state. Quantities as large as 100 liters (*e.g.*, the serum from several horses), or as small as 0.1 ml. may be processed. The material is frozen by immersion of its containing vessel in a mixture of acetone and solid CO_2 at -70 to $-80°$ C and immediately placed under high vacuum. Evaporation then proceeds at a rate sufficient to maintain the frozen state. The vapor is condensed in a suitable trap immersed in a cold bath of acetone-solid CO_2 mixture.

The processing of many liters in bulk is of value for purposes of storage and concentration. The processing of small amounts in individual containers is of value in that it makes possible the preservation and distribution of labile materials in any quantities. The apparatus described is capable of handling simultaneously any number of containers, from one to several hundred, each holding from 0.1 ml. to 500 ml. Upon completion of the process the containers are sealed off by fusing the glass exhaust tube with a torch, thus providing for storage in vacuum in individual sealed containers. All operations involved in the entire process are easily carried out with sterile technique.

Quantitative tests on complement and antisera kept at various temperatures indicate that the period over which they retain their original potency is greatly prolonged by this method of preservation. Thus, complement so processed has shown no demonstrable deterioration after 4 months at room and icebox temperatures, and diphtheria antitoxin and antipneumococcus sera have maintained their protective titer for the maximum period tested, namely 2 months, at 50° C. Tests are of course being continued for longer periods. The method makes available to the serological laboratory a pooled and stable complement, eliminating the necessity of daily bleeding and assuring a uniform product. It affords an improved means of preserving antitoxic and antibacterial sera, and a convenient means for their storage and distribution.

Finally, the process is making possible a wider exploration of the possibilities in the prophylactic and therapeutic use of human serum. A collection of human convalescent and adult sera preserved by this method has already been begun in Philadelphia. Clinical experience with these sera in measles prophylaxis has been favorable.

* Among those whose previous use of these conditions for preserving such substance we would acknowledge are Professor W. J. Elser of Cornell, Drs. L. F. Shackell, D. L. Harris, L. A. Rogers, Homer F. Swift, F. M. Huntoon and James Craigie.

Discussion

(Dr. E. G. D. Murray, Montreal.) I should like to ask Dr. Mudd whether he weighed very large quantities in doing the estimations of retained potency of this dry complement. I think in most laboratories we have to weigh very small quantities, and due to the error in weighing most of us do not get a straight line, as shown by Dr. Mudd. I should like to ask him what difficulties in the application of this work arise from the error due to weighing dry material.

(Dr. Lloyd Felton, Boston.) A question comes to my mind in a comparison of the dried and liquid complement. I could not tell from the chart whether or not Dr. Mudd meant that he added water to the dried material and kept it in that way. Of course most people interested in immune serum have dried it for years. I remember as a medical student I dried complement on filter paper and kept it for weeks at a time. The interesting thing in this paper is that there has been a method developed which can be made of commercial application in furnishing dried material, but I do not see how in the comparison between the dried and liquid materials there can be any advantage of the former over the latter. May I ask Dr. Mudd why the reconstructed complement is superior in keeping qualities in the icebox to the fresh complement bled and kept in the icebox?

(Dr. Mudd, closing.) In the tests shown complement was not weighed in the dried form. We redissolved the processed complement by making up to original volume with distilled water. Pooled complement preserved in the "lyophile" form at room temperature, and redissolved, has been found by Dr. Eagle to be identical in activity and keeping qualities with freshly pooled complement, within the limits of Dr. Eagle's method of titration (±10 per cent).

The method described does not endow sera with any activities they do not originally possess; it does allow for the preservation of sera, apparently for an indefinite time. Two hospitals which have used lyophile pooled complement for routine Wassermann tests have found it both economical and convenient; a complement of known and constant activity is thus made available for use at any time.

In the case of human adult or convalescent serum the method is of service in that it permits sera to be collected when available and utilized when needed.

There has been established at the Children's Hospital, under the direction of Dr. Joseph Stokes, Jr., what we are calling the Philadelphia Serum Exchange. Pooled adult human serum and convalescent serum from diseases in which passive immunization is practicable are collected from Wassermann-negative persons, passed through a bacterial filter as an extra precaution, and processed with sterile precautions. The sera are redissolved by addition of distilled water and injected intramuscularly. The sera so preserved have been used successfully for measles prophylaxis in the children's wards of five Philadelphia hospitals and in a considerable number of cases in private practice. Smaller numbers have been protected against chicken-pox. Pooled adult serum is also being injected, practically as a routine procedure, into patients entering the Children's Hospital, as a prophylactic against possible exposure to contagious disease before admission. An additional advantage of preserving serum in the "lyophile" form is that it permits concentration of the serum.

EXPERIMENTAL VACCINATION WITH B.C.G. B. J. Clawson, Minneapolis, Minn.

Abstract. Rabbits were vaccinated with 1 mg. of B.C.G. at 4 weekly intervals by the four following methods: (1) subcutaneously with living organisms; (2) intravenously with living organisms; (3) subcutaneously with heat-killed organisms; and (4) intravenously with heat-killed organisms. The results were studied with respect to safety and resistance.

Safety: The degree of safety was estimated from the presence or absence of tuberculous lesions in the lungs, liver, spleen and kidneys in from 3 to 16 weeks after the last injection of the vaccine, and from the presence or absence of allergy, as indicated by the Mantoux test. The lesions observed were microscopic and in no case showed any evidence of becoming progressive.

Lesions were found only in animals vaccinated intravenously with living B.C.G. and only in the allergic animals of this group. Allergy was present in a high percentage in the animals vaccinated by all the methods, except in those vaccinated intravenously with heat-killed organisms.

From the standpoint of lesions and allergy the method of administering the B.C.G. vaccine as heat-killed organisms intravenously carries with it the greatest degree of safety.

Resistance: Three weeks after the last injection of the vaccine another group of animals was injected intravenously with 0.01 mg. of a virulent bovine strain.

It was observed from the greater length of life in the vaccinated than in the non-vaccinated animals, from noting the less amount of involvement or absence of infection in the vaccinated animals, and from the absence of any resistance in the animals vaccinated with the timothy grass bacillus, that vaccination with B.C.G., living or heat-killed, gave a degree of specific resistance. While resistance was present in all four groups, it seemed to be greatest in the groups vaccinated subcutaneously with living B.C.G., and intravenously with heat-killed B.C.G.

The experiments seemed to show that efficient vaccination with B.C.G. may be safe and that, while allergy may be associated with resistance, as great or a greater degree of resistance against a tuberculous infection may be developed without a measurable amount of allergy. Vaccinating intravenously with heat-killed organisms seemed to be the best method to develop resistance with safety.

Discussion

(Dr. E. G. D. Murray, Montreal.) I think that we still have to be very cautious in drawing conclusions from data of this kind in applying this immunization to humans because, in the first place, relatively few animals were used in the experiments and, in the second place, it is dangerous to argue from animals to man. It has been shown by Stanley Griffith, who most of you know is a very cautious worker and who I know is rather interested in B.C.G. immunization and has had great hope of it, that although he can immunize calves, guinea pigs and rabbits, he has failed completely to immunize monkeys against tuberculosis, either bovine or human. On that account I think, in spite of the evidence of a lot of this kind of work which is accumulating, we still have to be very cautious before applying this method to human beings, even leaving out of consideration the possibility and probability of dissociation of this attenuated strain into a virulent phase, as several reliable workers have demonstrated.

(Dr. Esmond R. Long, Philadelphia.) The results presented by Dr. Clawson are convincing and are quite in accord with other experience on immunization with attenuated forms of living tubercle bacilli or with dead bacilli. We have always been able to secure some immunity, although not so good, with the old Saranac Lake strain, R I. I agree with Dr. Murray, however, that we should be very cautious in transferring these results to human beings. The situation as regards human tuberculosis is so different from that of experimental tuberculosis that we have to take special precautions in the interpretation of experimental results.

I should like to ask Dr. Clawson if he noticed any difference in the anatomical character of the tuberculosis in the controls and in those that had received the vaccination. We all know that we have at least two distinct kinds of human tuberculosis, the tuberculosis of childhood and the adult type. The point has frequently been made that we cannot expect B.C.G. to be very effective in the prevention of adult tuberculosis when we realize that adult tuberculosis develops in the presence of an old childhood tuberculosis. If a childhood invasion which is sufficient to make a calcified nodule in the lung is not sufficient to prevent the later development of tuberculosis, how can we expect that B.C.G. will do it? On the other hand, it seems to me that this type of immunization may be worth while in protecting against these progressive childhood infections which are responsible for a certain amount of our tuberculosis mortality.

(Dr. Clawson, closing.) I have only reported data on rabbits. The results seem to be as good, if not better, in protecting guinea pigs against the human strain.

In regard to the differences in the character of the lesions in vaccinated and unvaccinated animals I do not want to speak definitely, but I think there was more necrosis and a great many more organisms in the lesions in the non-vaccinated animals. In many lesions in the vaccinated animals it was impossible to find organisms at all. In most of the lesions in the non-vaccinated animals the organisms were distinct and could be seen by holding the tissue up to the naked eye.

The question which Dr. Long raised about the childhood tuberculosis having a protective effect against subsequent infection raises the question of just what degree of immunity such children do have as a result of just one lesion. I attempted to measure that to some extent by experiments. I made a series of animals allergic by giving them a large injection of B.C.G. subcutaneously, and then measured the concentration of the antibody by determining the complement fixation and agglutination titers. I then took another series of animals and vaccinated them intravenously with heat-killed organisms, and the titers were decidedly greater, which suggested that by vaccinating those that are allergic an increased immunity may be induced.

THE PATHOGENESIS OF ACUTE ANTERIOR POLIOMYELITIS. Maurice Brodie (by invitation), New York City.

Abstract. In order to determine whether or not the nasal cavities constitute the portal of entry for the virus of poliomyelitis and also to determine whether or not the virus travels along the olfactory nerves a bilateral section and partial removal of the bulb and tract was carried out on a series of *Macacus rhesus* monkeys by means of a transfrontal approach prior to intranasal administration of virus. The animals with cut olfactory nerves resisted intranasal inoculations

of the virus, in one experiment as many as 12, whereas the controls became paralyzed following a short incubation period after a single inoculation.

The nasal mucosa is innervated not only by the olfactory nerves, but also by branches of the fifth and seventh cranial nerves. In addition, virus can percolate from the nasopharynx to the tonsils with its intact nerve supply. Yet, upon cutting the olfactory tract no infection occurred, indicating clearly that the first cranial nerve is the only one of the nasopharynx that can transmit the virus of poliomyelitis from the nasopharynx to the central nervous system. Over a period of 3 weeks 2 experimental animals received 12 intranasal inoculations, of which considerable must have dribbled into the gastro-intestinal tract. This observation discounts the gastro-intestinal tract as the portal of entry.

When animals with severed spinal cord but closed dura and an intact hematogenous system and flow of spinal fluid were injected into the upper or lower segment the virus failed to pass the gap in the cord, showing that the virus spreads along nerve tracts and not by the spinal fluid or blood. Inasmuch as the perineural lymph spaces of the olfactory nerves continue into the subarachnoid space and the nerve fibers into the rhinencephalon the virus must travel along the nerve fibers. A correlation between the infectivity of the central nervous system of monkeys, at the acute stage of the disease, with the amount of nerve cell destruction and also the loss of infectivity with removal of neurones, indicates that most of the virus is lodged in the neurones.

Experimental poliomyelitis, then, is entirely neurotropic; the virus travels along the olfactory nerve fibers to the central nervous system where it is propagated along the nerve tracts. Since only the olfactory nerve of the nasal cavity can carry the virus and because no infection was obtained when large amounts of virus reached the gastro-intestinal tract the portal of entry must be the nasal cavities.

ST. LOUIS ENCEPHALITIS — EXPERIMENTS ON PATHOGENESIS AND IMMUNITY. Leslie T. Webster and (by invitation) George L. Fite, New York City.

Abstract. Susceptible mice given an intranasal instillation of the virus of St. Louis encephalitis develop, after a 5 to 6 day incubation period, an encephalitis that terminates fatally in 7 to 10 days. Their bloods and spleens rarely contain detectable virus at any stage of the infection. Their brains, however, show virus 2 days after infection and constantly thereafter, in amounts increasing to a maximum by the 6th day, when signs of disease first appear. Lesions in the anterior region of the olfactory lobes are demonstrable on the 3rd day after infection. On the 4th day the lesions extend to the lobus piriformis, and on the 5th day to the cornu Ammonis.

Mice given an intraperitoneal or subcutaneous injection of 1000 lethal doses of the virus do not develop encephalitis but remain well. The injected virus is detectable in the blood, however, in considerable quantity within 10 minutes, but disappears after a few hours, depending on the size of the infecting dose. In the spleen virus persists for at least 7 days.

These mice injected intraperitoneally or subcutaneously become immune, within 7 days, to 1000 intranasal or 1,000,000 intracerebral lethal doses. Indeed, a single subcutaneous or intraperitoneal dose as small as 0.000001 gm. brings about this high grade immunity, persisting unchanged for 5 weeks and doubtless much longer.

SEROLOGICAL RELATIONSHIP OF ST. LOUIS AND JAPANESE ENCEPHALITIS. Leslie T. Webster and (by invitation) George L. Fite, New York City.

Abstract. The recent outbreak of encephalitis in and about St. Louis, Mo., resembles epidemiologically and clinically the encephalitis in Japan designated Type B. A further comparison of the two diseases has now been made by means of a serological test based on the fact that the virus of the St. Louis disease mixed with serum from convalescents and injected intracerebrally into mice is specifically neutralized. Fifteen sera from cases in Japan have been tested — three from persons with encephalitis in August, 1924, and twelve from persons with encephalitis in August, 1933. None of the sera showed any protective action against the St. Louis virus.

Discussion

(Dr. Ralph D. Lillie, Washington.) I should like to ask what proportion of the sera from the St. Louis outbreak would protect against the virus which came from the same outbreak.

(Dr. Howard T. Karsner, Cleveland.) This work has justifiably attracted widespread attention. The large volume of material presented in a short time has left me uncertain as to whether or not Dr. Webster and Dr. Fite have established the identity of these viruses as separate and distinct from others, for example, that of herpes.

(Dr. T. M. Rivers, New York City.) I am certain a few words can clarify the situation for Dr. Karsner. In the first place it may be rather difficult for those not familiar with viruses to understand how two virus diseases of the central nervous system may present the same clinical, epidemiological and pathological picture, and yet be quite different serologically. The St. Louis encephalitis and the Japanese summer encephalitis are similar, if not identical, clinically and pathologically but the active agents involved are immunologically different. The brain responds to injury in a limited number of ways. Consequently, different etiological agents can produce the same clinical and pathological picture. There is no doubt of the fact that the sera which came from Japan do not neutralize the virus recovered from the St. Louis epidemic. Among the diseases of lower animals one finds good examples of the state of affairs about which I have just spoken. In horses, for instance, an encephalomyelitis occurs in the East and in the West. The disease in the two localities is similar clinically but different immunologically. The same is true of foot-and-mouth disease: different strains of virus produce the same clinical and pathological picture, but differ immunologically. Furthermore, in vesicular stomatitis there are two strains of virus which cause the same clinical picture but differ immunologically. One does not need to illustrate the point in question in more detail. It is not surprising, therefore, that in human beings there are virus diseases which resemble each other very closely and yet are caused by active agents immunologically different.

(Dr. Lloyd Felton, Boston.) Dr. Rivers has answered the question I was going to ask, but I should add this comment — that occasionally we need a little mental stimulation when it comes to medical research. Some people do; others don't, but I do. When Dr. Webster came to Boston and gave his paper I was sure if Paul de Kruif had been present he would have made a picture of it. To me it was a beautiful piece of work done in such a short time and in such

conclusive fashion. Here is the picture of an epidemic in the West and the doctors running around and trying to do everything they can to help. Dr. Webster goes out and with the greatest spirit of coöperation with the Westerners he detects the virus and injects it into mice. He gets it out and follows it, as the picture shows you. It was a lot of work, requiring a lot of patience. To me it is such a pretty thing that although I am a pessimistic optimist it does give a worthwhile slant on medical research, and really doing things that can be done in a clear-cut fashion. I think Dr. Webster and the people who coöperated with him deserve a great deal of commendation for the very excellent way they have conducted this research.

(Dr. Maurice Brodie, New York City.) Regarding what Dr. Fite has brought out concerning the Japanese epidemic being different from the St. Louis epidemic, there is a possibility of the same thing in polio, as pointed out by Australian workers. In studying human strains we have found immunological differences between two recently isolated strains and the monkey passage virus which we are studying. Whether there is no relation whatever between them, or whether it is merely quantitative we cannot as yet say, but we know there is a difference. We have done some work with the encephalitis virus, and the first question was whether it was herpes, and I think Dr. Fite pointed that out pretty well, but we have done other work on it. The fact that you cannot infect guinea pigs speaks pretty definitely against its being herpes. We took a series of forty normal sera, and whereas over 50 per cent of normal individuals show neutralizing substances to the herpes virus no one showed neutralizing substances for this virus.

(Dr. Fite, closing.) In regard to the percentage of the St. Louis cases that neutralized the virus I am sorry I have not the figures here, but over 90 per cent of those sera that were taken more than 10 days after the onset of the disease did neutralize it. Those taken earlier for the most part did not.

Dr. Brodie has answered pretty well the argument against this virus being the herpes virus. It does not infect guinea pigs or rabbits. Inoculation of the eye, skin or brain of a rabbit produces no fever, no restlessness, no sign of herpes whatever. Herpes sera fail to neutralize the virus in any degree. There are many of the same sort of reasons for believing it is not identical with the virus of equine encephalomyelitis or vesicular stomatitis. We ourselves, who happen to be familiar with the virus of louping-ill, know there is no cross-neutralization between the sera of either one with the other virus.

THE GROWTH OF CAPSULAR SUBSTANCES INDEPENDENTLY FROM THE BACTERIA. Louis Dienes, Boston, Mass.

Abstract. Certain bacterium strains produce in excessive amounts capsular substances and their growth is the cause of such phenomena as the ropiness of beer, mucoid fermentation of dough, and so on. We studied subtilis strains showing this property and observed that on saccharose agar plates the mucoid extrabacterial material grows out from the colonies to a considerable distance (up to 20 mm.) on the surface of the agar, forming a halo around the colonies. The halo contains no bacteria and transplants of it on the usual media remain sterile. On saccharose-containing media the transplants have been cultivated in 4 generations as a transparent material in which bacteria were not reproduced. In preparations stained with flagellar staining methods the capsules of the bacteria appear as a dense, hair-like growth of fine filaments. The capsules

of pneumococcus and of other different bacteria appear to be similar. The extrabacterial substance in the subtilis colonies consists mostly of the above mentioned fine filaments. There are many degenerating bacteria in the culture which often show polar staining, and many polar bodies are lying free among the bacteria. In preparations from the halo, stained with flagellar staining methods, we found different morphological elements: long, fine filaments which represent probably the element on which the expanding of the halo depends; short, very fine filaments connected with granules; small, round or oval, spore-like bodies which sometimes form with the filaments asteroid figures; and broken-off parts of thicker filaments which sometimes grow out from the spore-like bodies. The morphological elements of the halo are different from the elements of any known bacterial culture but present close similarity to the elements found in the cultures of the viruses of *pleuropneumonia bovis* and agalactia. The growth phenomena and the definite morphological structure of the halo furnish convincing evidence that the production of the halo represents the growth of living elements different from the usual bacterial forms. The structures which are regarded as a capsule around the bacterium may assume a quite different biological significance. As every spore from a heated spore emulsion reproduces the halo it is probable that the elements of the halo are derived from the bacteria.

Discussion

(Dr. Marcus W. Lyon, South Bend.) I should like to ask Dr. Dienes how he stains these structures. They look like flagella in some instances and in others are quite different.

(Dr. Dienes, closing.) The filamentous structures in the culture and the capsule of the pneumococcus are stained best by Loeffler's method, while the elements in the halo are stained best by Zettnow's method. These filamentous structures are certainly different from flagella, because they can be stained beside each other in the same preparations.

THE PROTEASES AND ANTI-PROTEASES OF PLEURAL EXUDATES. Charles Weiss and (by invitation) E. J. Czarnetzky, San Francisco, Cal.

Abstract. Not received.

TISSUE REACTIONS IN IMMUNITY: TISSUE-ANTIGEN REACTIONS IN PROTEIN-IMMUNIZED RABBITS. Reuben L. Kahn and (by invitation) Elizabeth L. McDermott, Ann Arbor, Mich.

Abstract. When specific protein is injected into some fixed tissue, such as the skin or muscle, of a protein-immunized rabbit the protein is localized at the site of injection and an inflammatory response is soon noted. It is believed that the tissue combines with the protein, thereby anchoring it and preventing it from diffusing throughout the body, and that the inflammatory response tends to wall off the injected area and destroy the protein. With a standard method of immunization a given tissue possesses a combining capacity for a certain quantity of protein only. When this quantity is exceeded uncombined protein finds its way to the other tissues of the body. Also, different tissues of protein-immunized rabbits possess different capacities for combining with the protein, the cutaneous and peritoneal tissues possessing combining capacities approximately ten times as great as those of *in vivo* plasma and of muscle and brain

tissue. If a protein-immunized rabbit is given an intravenous injection of the specific protein and soon after the same protein is injected into the skin, no local inflammatory response will be noted. As a result of the intravenous injection the animal is disimmunized (desensitized) temporarily.* The term disimmunization is preferred to desensitization, which is used to express clinical conditions unrelated to these experiments. By disimmunity is meant the reverse of the immune state. Only an immune animal can be disimmunized, hence disimmunity is not synonymous with susceptibility or non-immunity. The tissues in the disimmunized state perhaps become "saturated" with the protein and thereby lose their capacity to combine with additional protein.

These quantitative aspects of the antigen-combining capacities of the tissues of protein-immunized rabbits are indicated by a method devised in this laboratory.† The method utilizes horse serum as the immunizing reagent and modified horse serum in the form of antitoxin with its specific toxin as the reagents for measuring the tissue changes as a result of the immunization with horse serum. Thus, if into the skin of a horse serum-immunized rabbit are injected 50 M.L.D. of diphtheria toxin, and simultaneously 750 units of horse serum antitoxin are injected subcutaneously in another area, the rabbit will succumb, indicating that the subcutaneous tissue combined with the antitoxin, thus permitting the toxin which diffused through the body to exert its lethal action unhampered. If 1000 units of antitoxin are injected the rabbit will survive; some uncombined antitoxin presumably escapes from the area of injection and neutralizes the toxin *in vivo*. When a horse serum-immunized rabbit is injected intravenously with a considerable amount of horse serum the rabbit becomes temporarily disimmunized; the skin loses its capacity to combine with antitoxin and 20 units of this reagent will be found sufficient to save this animal from 50 M.L.D. of toxin, similar to the amount of antitoxin required to save normal rabbits.

It is believed that these findings have a bearing on immunity to bacteria, emphasizing particularly the quantitative nature of the immune state of the tissues. The skin, for example, may have a reacting or combining capacity for staphylococci of certain virulence, sufficient to prevent these organisms from permeating into the deeper tissues of the body. This immune manifestation apparently is broken down if the quantity of organisms exceeds the limit of the combining capacity of the skin. The fact too that the skin possesses this combining capacity to a greater extent than muscle or brain tissue would indicate that the property of the skin to prevent the spread of these organisms into other tissues of the body is greater than that of muscle or brain tissue. In a condition where the organisms circulate in the blood stream in considerable numbers we are undoubtedly dealing with a phase of disimmunity. Whereas in the immune state the host is able to keep the infecting organisms localized, in the disimmune state the organisms tend to become widespread throughout the body. The host has the upper hand over the organisms in the immune state, while the organisms have the upper hand in the disimmune state. It should be added that under the conditions of the experiments described the disimmune state is rapidly followed by an immune state usually of a higher level than before disimmunization, indicating that the disimmune state serves as a stimulus to the animal to build up its immunity.

* Kahn, R. L., *J. Bact.*, 1934, **27**, 92.
† Kahn, R. L., *Science*, 1934, **79**, 172.

TISSUE REACTIONS IN IMMUNITY: TISSUE-ANTIGEN REACTIONS DURING PERIOD OF INCUBATION. Reuben L. Kahn, Ann Arbor, Mich.

Abstract. The period of incubation in these experiments refers to the interval between the time of injecting a protein into a rabbit and the development of the capacity of the skin specifically to react to a second injection of the protein. Little is known regarding the immunological changes in the body tissues of the rabbit during this period of incubation, which is believed to extend for about a week or more. By means of the antitoxin method utilized in the studies presented in the preceding paper it was possible to establish that immunological changes begin to take place in the skin of the rabbit as early as 48 hours after an injection of protein. If a rabbit is given an immunizing injection of horse serum and 2 days later is injected with 50 M.L.D. of diphtheria toxin intracutaneously, and simultaneously in another area of the skin with 20 units or in some cases with 25 units of horse serum antitoxin, the animal will succumb from the toxin. Normal rabbits succumb from 50 M.L.D. toxin when only 15 units or less of antitoxin are injected into the skin, but are saved by 20 units. It is believed that the skin of the 2 day previously injected rabbits develops as a result of the injection a slight capacity to combine with antitoxin, which is essentially specific antigen. Hence, 20 or 25 units of antitoxin are not sufficient to save them from toxin death.

It was further observed that many factors play important rôles in the determining whether tissue changes will take place during this short incubation period or not. Preliminary experiments indicate that the quantity of horse serum injected and whether it is undiluted or diluted with salt solution; the route of injection; the age of the rabbit, especially whether it is in a growing or in a mature state; also the non-specific immune state of the rabbit — all these and undoubtedly other factors affect tissue changes during this period. A relatively large dose of horse serum, injected intracutaneously or subcutaneously, may prolong the incubation period, whereas a small dose of the protein solution is likely to shorten the incubation period and thus hasten the appearance of detectable immune changes in the skin. When the horse serum is injected intravenously, thus far no detectable skin changes have been observed 2 days after the injection. Mature rabbits show more marked tissue changes during the period of incubation than young rabbits. Non-specific immunity also tends to hasten tissue changes. Beginning with 3 days after an immunizing injection of horse serum specific tissue changes show a gradual increase. No attempt has thus far been made to determine tissue changes in less than 48 hours after the injection.

It would appear from these studies that the tissues of an animal during the incubation period following the entrance of bacteria into the body undergo important immunological changes. The extent of these changes may be a determining factor as to whether or not the organisms will be prevented from establishing an infection. Indications are also that the smaller the quantity of organisms gaining entrance the more rapid is the immune tissue response, while with a larger quantity of organisms the oncoming of immunity is likely to be delayed. The general belief that small doses of infecting material tend to immunize, while large doses tend to bring about infection, appears to be substantiated by these studies with protein. That the tissues of mature animals possess greater immune capacity than those of young animals is also of interest. It is hoped that extensive studies of these factors governing tissue im-

munity during the incubation period will throw light on the immune mechanisms of the body tissues during this period and will open the way to special means of stimulating the immune response of the tissues during this same period in infection.

Discussion

(Dr. Stuart Mudd, Philadelphia.) I have been impressed with the recent papers of Dr. Arnold Rich in the field of allergy and immunity. It seems to me that he has advanced the subject importantly by showing that allergy and immunity can be separated. I take it from Dr. Clawson's paper this morning that he also has been able to distinguish and separate immunity and allergy in tuberculosis, the field in which these two conditions have most often been regarded as perhaps inseparably associated. If I understand Dr. Kahn correctly he is introducing the term "disimmunization" and defining it in terms of removal of allergy. It seems to me that introduction of a new term into an already confused terminology is always a doubtful step; in particular the question arises in my mind as to whether Dr. Kahn's term "disimmunization" serves to clarify or merely further to confuse this subject.

(Dr. Louis Dienes, Boston.) From the interesting observations of Dr. Kahn I was mostly interested in the early appearance of specific response. I have studied, together with Dr. Tracy B. Mallory, the first appearance of hypersensitiveness and we found that either in rabbits, guinea pigs or in man it is possible to obtain definite skin reactions on the 4th day after the immunizing injection. We found, furthermore, that the early manifestations of the specific response are qualitatively different from the later manifestations. The early skin reactions do not start to develop with an exudation like the later reactions, and infiltration with mononuclear cells is the most pronounced feature of them. This type of reaction is present on the 4th, 5th and 6th days after treatment; later — and always in passively sensitized animals — the skin test produces a different type of reaction in which exudation plays the most important part. So the mechanism of the specific reactions which the antigen produces at the site where it comes in contact with the tissues is markedly different in the early and later stages of immunization. We could not obtain direct evidence concerning the rôle which the early phase of the specific response plays in the healing of disease, but it seems probable that this rôle is important. The late development of antibodies in disease and the observation that the introduction of antibodies, except in the first few days of disease, exert hardly any influence on it show that the healing cannot be explained simply by the production of antibodies. Such observations as the development of bone lesions or orchitis after the healing of typhoid fever point to the fact that the antibodies are not the only factor in the healing process and the local tissue resistance at the site of the lesions plays an important rôle in it. It is not impossible that the early phase of the specific response, which is purely a tissue response, is more important for the healing than the production of circulating antibodies. The study of the early phase of specific response might give us important new information and it seems to me that Dr. Kahn has indicated a new and a very fine method for this study.

(Dr. E. G. D. Murray, Montreal.) I think the question of the difference between allergy and immunity might lead to an interminable discussion. However, it seems more profitable at the moment to regard allergy as just a stage in the

production of immunity. This work of Dr. Kahn's has a very direct application. The astonishing thing to me is that the peritoneal route in the immunized animal should hold up the antigen to such an extent, because one would have expected on what has been done before that the absorption of antibody introduced by the intraperitoneal route would be only second to the intravenous route. The application of this work is in the administration of immune serum. I have been privileged, very much privileged, by the courtesy of the Connaught Laboratory, to use staphylococcus antitoxin therapeutically during the past year, and it has been very surprising in patients who have some degree of, let us say, sensitiveness to horse serum, what an astonishing amount of that antitoxin has to be administered by the intramuscular route, compared with patients who have had no history of previous administration of horse serum and who showed no degree of what is called allergy. The intravenous route is certainly the most advantageous route, but the serious reactions which followed the administration of serum in certain cases place the patient in a position of very grave danger. Therefore other routes are desirable, and in the administration by the intramuscular route we have found it necessary to "disimmunize" the patient. I should like to ask Dr. Kahn, mainly for my own guidance, how long the stage of "disimmunization" in these animals lasts, because at times with repeated administrations of antitoxin one has to face that problem.

(Dr. Kahn, closing.) I am thankful to Dr. Mudd for bringing up the question of the relation between allergy and immunity. It is true, as Dr. Murray stated, that if we entered extensively into a discussion of this subject we would never get through. It is important, however, to emphasize our different points of view from time to time so that we may all keep this subject in mind. The question under consideration is in reality whether a specific inflammatory response is immunological or allergic in nature. If immunological the inflammation must be interpreted as being protective; if allergic the inflammation must be interpreted as some condition opposite to protection. Pathologists have long ago defined inflammation as a defensive mechanism. If this definition is to stand it seems to me that we must accept the specific inflammatory response in an immunized animal as immunological in character. Therefore, when, by some special means we rob the tissues of the capacity to produce a specific inflammatory response we produce a condition which is the reverse of the immune state, namely, we disimmunize the animal.

To illustrate — a given amount of horse serum, such as 1 cc., is injected subcutaneously in a rabbit. The horse serum soon diffuses from the area of injection and the greater portion is gradually eliminated through the kidneys. Suppose the same quantity of horse serum is injected subcutaneously in the same rabbit some 2 weeks later. The injected horse serum does not diffuse from the area of injection; it remains localized and an inflammatory response soon becomes visible in that area. The inflammation apparently tends to wall off the injected area and destroy the horse serum by proteolysis. But the skin can be robbed of the capacity to destroy the horse serum locally by means of an inflammatory response. All that is necessary is to inject the rabbit intravenously with horse serum. When this is done it may be that all body cells become saturated with the horse serum. Therefore, when this serum is injected into the skin the cells are incapable of combining further with it and of bringing about an inflammatory response. Following the intravenous injection of the horse serum the rabbit becomes disimmunized for a certain number of hours or for a day or two, depending on the quantity injected. The disimmunized state is

followed by the immune state when the skin will again respond with local inflammation to a cutaneous injection of horse serum. It should be recalled that the term desensitization is employed by clinicians to designate the return of allergic patients to normality. Obviously the horse serum-immunized rabbit which has been injected intravenously with horse serum has not thereby been brought to the normal state.

We are all familiar with the studies of local tissue immunity of Dr. Cannon and his associates in the University of Chicago. If tissue responses are basically allergic it seems difficult to understand how we can have local tissue immunity. I have the highest regard for the work of Dr. Rich and Dr. Clawson, but I regret I cannot agree with their interpretation. Dr. Murray raises an important question. The disimmunized state is of very short duration, an animal apparently making every effort to return to the immune state.

AN EFFECT OF ALTERING THE pH OF THE REACTANTS IN THE PRECIPITIN REACTION. James T. Culbertson (by invitation), New York City.

Abstract. Not received.

THE BACTERIOLOGICAL DIAGNOSIS OF ACTINOMYCOSIS. P. H. Greey (by invitation), Toronto, Canada.

Abstract. The clinical data on 20 cases of actinomycosis which occurred at the Toronto General Hospital in 2 years, representing a four-fold increase in incidence, was presented. Practically all of these cases were diagnosed bacteriologically and the procedure used routinely in making such a diagnosis was described.

BACTERIAL VARIATION IN PNEUMOCOCCUS AND STREPTOCOCCUS HEMOLYTICUS. M. H. Dawson (by invitation), New York City.

Abstract. Studies in variation in pneumococcus have shown that this bacterial species possesses a third and distinct variant form in addition to the currently accepted S and R forms. The characteristics of this third variant form are described in detail and the interrelationships between the three chief variant forms are considered.

Studies in variation in *Streptococcus hemolyticus* have demonstrated that this species also possesses three chief variant forms: (1) M (mucoid), (2) S (smooth), and (3) R (rough). Attention is particularly directed to the mucoid form. A comparison of these three chief variant forms of *Streptococcus hemolyticus* with those of pneumococcus reveals a remarkable parallelism in their salient characteristics.

A review of the subject of variation in a wide variety of bacterial species reveals that the general phenomenon of variation fits into a more or less orderly and uniform pattern. The studies on variation in pneumococcus and *Streptococcus hemolyticus* show that variation phenomena in these species conform to that general pattern.

Reference is made to the desirability of adopting a uniform terminology to describe corresponding variant forms in various bacterial species.

Discussion

(Dr. Charles Weiss, San Francisco.) May I ask what is meant by neopeptone?

(Dr. Dawson.) Neopeptone is a digest of casein which is now available commercially from Difco Laboratories, Detroit. Perhaps Dr. Avery will say a word about it.

(Dr. O. T. Avery, New York City.) The neopeptone referred to is a product, the preparation of which is based on the results of studies carried out in our laboratory by Dr. Dubos, who demonstrated the presence in various commercial peptones of substances which are bacteriostatic in the oxidized form. The removal of these toxic products by chemical methods enhanced the growth-promoting properties of media containing the purified peptones. Dr. Dubos showed further that a peptic digest of casein was devoid of bacteriostatic substances and that the use of this product in culture media facilitated the growth of minute inocula of the more difficultly cultivable microörganisms.

THE PATHOGENICITY OF THE GENUS BACTEROIDES. D. C. Beaver, Rochester, Minn.

Abstract. The genus *Bacteroides*, as defined by Castellani and Chalmers, and by Bergey's Manual of Determinative Bacteriology, includes certain obligate anerobic, motile and non-motile rods which do not form endospores. French and German investigators have emphasized the importance of this group of microörganisms, stating that they are a frequent cause of suppurative and gangrenous infections of man. Elsewhere in the literature they have been described only rarely.

Veillon and Zuber in 1898 were apparently the first to describe and isolate anerobic organisms of this type. They obtained several species from cases of appendicitis and other suppurative conditions, to which they gave the names *B. ramosus, B. serpens, B. fragilis, B. furcosus* and *B. fusiformis*. Hallé confirmed this work by isolating similar forms from the vaginal flora and from suppurative conditions about the female genital tract. He introduced a new species and named it *B. funduliformis*. Rist and also Guillemot, Hallé and Rist recovered similar species from pulmonary abscesses and putrid empyema. Cottet described them as the cause of phlegmonous periurethral infections of the male. Harris and Norris found types similar to those described by Veillon and Zuber in association with hepatic suppuration. The literature of the subject has been reviewed recently by Teissier, Reilly, Rivalier and Stefanesco (1931), and by Cohen (1932). Teissier and associates placed particular stress on the occurrence of bacteremia in the course of infection with *Bacteroides funduliformis*. They reported 4 cases. In Case 1 there were liver abscesses with apparently secondary abscesses of the skin and kidney. The illness in Case 2 began with sore throat; purulent pleurisy followed. The onset of illness in Case 3 was also with sore throat; purulent pleurisy with some involvement of the lung supervened. Case 4 also had onset with sore throat; secondary abscesses of the sternoclavicular and right sacro-iliac regions and right groin developed. Cohen studied the bacteriology of abscesses of the lung, and concluded that anerobic organisms of the type under discussion were the usual cause.

Of equal importance are the observations of Distaso, Debono, Teissier and Eggerth and Gagnon, that members of the genus *Bacteroides* are abundantly present in the normal human intestinal flora.

The present study confirmed the work of previous investigators. *Bacteroides funduliformis* and other species were found to be important as etiological agents in (1) infections of the male urinary tract; (2) ulcerating carcinomas of the colon; (3) perirectal abscesses; (4) suppurative appendicitis; (5) pylephlebitis; (6) abscesses of the liver; and (7) abscesses of the lung. In most instances there was demonstrable bacteremia. Infections were characterized by suppuration usually with abscess formation; sometimes there was gangrene. Experimental inoculation of guinea pigs and rabbits proved that the microörganisms were virulent for these animals; experimental abscesses similar to those observed in the human resulted. Infection of man by *Bacteroides funduliformis* and other species may provoke a profound toxic reaction, and in many cases the prognosis is extremely serious.

Discussion

(Dr. E. G. D. Murray, Montreal.) I should like to ask Dr. Beaver if he has any reason to account for the fact that he has found so few of these organisms in the fourth class, the female urogenital tract, considering that he has found such an unusual number of cases of infection in the other situations which he has described. In our experience *Bacteroides* can be isolated from the lochia of practically all women postpartum. We have isolated quite a variety of them, which we have considerable difficulty in placing. Among others we have a pigmented form which produces a dense black pigment and has some properties resembling melanin. Considering the great frequency with which these organisms are present in the human vagina it is surprising that there are not more serious conditions such as Dr. Beaver has described. I should like to know if he can explain that. I agree with him that they need a richer medium than is ordinarily described as being necessary for their growth. Usually they won't grow without blood or serum.

(Dr. Beaver, closing.) In reply to Dr. Murray I would state that the only reason I have not found *Bacteroides* in infections of the female genital tract is that the material which I have studied is routine material, and I happen to have access more to colon material than any other type. Probably if I had the same access to infections of the female genital tract, I would find *Bacteroides* as frequently as Dr. Murray and others have.

INCLUSION BODIES IN THE SALIVARY GLANDS AND LIVER OF MICE AND RATS. Juanita Thompson, Washington, D. C.

Abstract. This study was concerned with the spontaneous occurrence of a number of intracellular abnormalities in the salivary glands and liver of mice and rats. Some were found in relation to a highly fatal spontaneous epizoötic in which many of the animals presented gross manifestations closely simulating the so-called cutaneous or generalized type of infectious ectromelia. In a selected group of 25 animals inclusions were only demonstrated satisfactorily in five of the livers. The inclusions occurred in both the cytoplasm and the nucleus. The other intracellular formations seen were found in apparently healthy animals. In the mucous acini of the submaxillary glands in both mice and rats large inclusion-containing cells were found in a considerable number of the animals. These histological structures had many features in common with the inclusions associated with guinea pig submaxillary virus disease. In a considerable number of cases unusual intranuclear changes were also observed

in the acinar cells of the facial portion of the parotid glands of apparently healthy rats and in the hepatic cells of seemingly normal mice. The changes were comparable to the intranuclear inclusions reported by Findlay in the livers of a strain of apparently healthy mice. The intracellular formations seen in both rats and mice lead one to believe that at least in some cases their presence suggests latent virus.

PSEUDO SKIN REACTIONS AS A COMPLICATING FACTOR IN THE INTERPRETATION OF THE DICK REACTION BEFORE AND AFTER IMMUNIZATION AGAINST SCARLET FEVER. M. L. Menten and (by invitation) C. G. King and H. H. Finlay, Pittsburgh, Pa.

Abstract. Results of immunization against scarlet fever previously obtained by us in older individuals (doctors and nurses) indicated that in many of these despite 5 or even 6 immunizing doses of toxin many persistently remained Dick-positive, but were apparently not susceptible to scarlet fever. It appeared as if the skin of such individuals had acquired a sensitivity to certain proteins derived from the streptococcus.

In order to test such a hypothesis 112 individuals between the ages of 10 and 19 years, who gave positive skin reactions to Dick commercial toxin and specific toxin partially purified by us, were skin tested before immunization with the following 3 ancillary tests, *viz.*, toxin boiled 1½ hours, dilute antitoxin, and toxin neutralized with antitoxin. They were then given at weekly intervals 5 immunizing doses. On retesting the following results were obtained: 55, or 49 per cent, were negative to Dick toxin and negative to our toxin; 34, or 30.4 per cent, were negative to Dick toxin and positive to our toxin; 23, or 20.5 per cent, were positive to Dick toxin and positive to our toxin. The 57 who showed positive reactions were then given the 3 ancillary tests. Fourteen of the group of 23, who after immunization were still positive to both tests, were originally negative to boiled toxin and through immunization had acquired a positive reaction to boiled toxin. Immunization procedures had apparently rendered the skin more sensitive to protein reaction. Further evidence of this phenomenon was also noted in the acquisition of sensitivity to antitoxin following immunization. Analysis of the other group of 34 gave similar data.

We believe the above may help to explain some of the discrepancies reported by investigators of immunization against scarlet fever.

HEREDITARY VARIATIONS IN THE BLOOD CYTOLOGY OF NORMAL RABBITS. Albert E. Casey, P. D. Rosahn, C. K. Hu (by invitation) and Louise Pearce, New York City.

Abstract. Studies in the laboratory of the Rockefeller Institute for Medical Research have demonstrated wide variations in the blood cell formulae of normal rabbits comparable in magnitude with variations in coat color, size, body and organ weights and other constitutional factors. Except for minor seasonal fluctuations these differing blood cell formulae were found to be fairly stable during conditions of health and vigor and to be closely related to the natural resistance of the rabbit host to several experimental and spontaneous diseases. Further studies on standard bred stock have revealed that the differences in the blood formulae among normal rabbits are largely inherited differences, and studies on the transmission of such characters are now being

made. The most striking evidence of inheritance was found in the values for the basophiles, lymphocytes, red blood cells, and the hemoglobin, factors which were found to be reliable indices of the natural resistance of rabbits to inoculation with a transmissible malignant tumor and with the spirochete of syphilis.

THE FATE OF INJECTED MARKED HOMOLOGOUS LEUKOCYTES IN THE GUINEA PIG. John Ungar and G. Randolph Wilson (by invitation), Pittsburgh, Pa.

Abstract. The purpose of the experiment was to observe the fate of marked homologous leukocytes injected into the circulation of the guinea pig. Leukocytes were obtained by the production of a chemical peritonitis with 5 per cent aleuronat. Carbon and a protein substance, carmine, were the suspensoid dyes injected for the purpose of marking the cells for later identification. Guinea pig serum injected together with the dye was shown to enhance phagocytosis both *in vivo* and *in vitro*. Several hours were allowed for phagocytosis. The abdomens were then opened and the cellular exudate removed and placed in a sterile flask which was kept in the dark at body temperature. It was then filtered. The filtrate was clear of free particulate matter and consisted of a pure suspension of leukocytes, approximately one-half of which contained phagocytosed dye particles. The cells were concentrated by slow centrifugalization and proved viable by their ability to phagocytose a contrasting dye *in vitro*. They were then injected intracardially in some animals and into the portal vein of others. Histological studies were made after various time intervals. The carbon-containing leukocytes were found to be concentrated in the lungs, free in the air spaces. Carmine-containing cells did not have a consistent distribution. Some appeared in the lungs to the exclusion of other organs, while in others destruction occurred chiefly in the liver.

Discussion

(Dr. N. C. Foot, New York City.) I do not think that any of us are so enthusiastic about the origin of the mononuclear cells in the intra-alveolar spaces from the blood as to maintain that all of them come from that tissue, but a piece of work like this is always very gratifying to those of us who have that belief. The mononuclears are so ubiquitous, they are such restless cells, and they move to and fro with such rapidity that it is very difficult to keep any track of them at all. This method of (shall we say bird-banding?) is a very satisfactory way of keeping track of them. There is one possibility, that these cells concentrate in the lung as they do on account of their large load of pigment. In other words, they act very much as pigment or particulate matter does, and I think it would be interesting to follow the experiment along and see if the cells remain viable in the lung for any length of time and whether or not they wander back from the alveolar cavity in the lymphatics.

THE CHEMOTROPIC ATTRACTION OF HUMAN LEUKOCYTES BY MICROÖRGANISMS AND VARIOUS SUBSTANCES. Morton McCutcheon, Harold M. Dixon (by invitation) and Edward B. Krumbhaar, Philadelphia, Pa.

Abstract. The chemotropic response of human polymorphonuclear leukocytes to different types of microörganisms was evaluated through experiments *in vitro*. Under the microscope the net distance was measured by which each cell approached a clump of bacteria, and this distance was divided by the total

length of the path of the cell. The resulting ratio is a measure of the chemotropic response, having as extreme values +1.00 if the cell moves directly toward the bacteria and −1.00 if the cell moves directly away from them. With staphylococci, streptococci, pneumococci, typhoid and tubercle bacilli, *Micrococcus tetragenus* and certain yeasts, mean ratios ranged only from +0.73 to +0.86, indicating approximately equal attraction under these conditions. With the yeast *Torula histolytica* the ratio was +0.57. Control leukocytes, wandering in fields free from known chemotropic influence, gave a value of +0.07. With substances other than bacteria a wide range of values was obtained: gelatin 0.00; dried blood +0.18; dried leukocytes +0.25; starch paste +0.71.

Discussion

(Dr. Louis Dienes, Boston.) Though it is well known, I should like to mention that the tubercle bacilli attract polymorphonuclear leukocytes also in the tissues, as in the diagram shown to us. The infiltration with mononuclear cells and the tubercle formation is a later reaction due, according to our observations, to the development of hypersensitiveness.

STUDY OF THE BONE MARROW IN APLASTIC ANEMIA. C. P. Rhoads and (by invitation) D. K. Miller, New York City.

Abstract. Twenty-two cases of so-called aplastic anemia have been studied both clinically and pathologically. These cases were divided into four subgroups according to the pathological changes observed in the bone marrow. Sternal marrow removed at biopsy was studied in all except 3 cases. In the first, or "classical aplastic" group, the marrows showed a striking reduction of total numbers of cells as well as an interference with maturation. In the second, or "immature" group, the marrows were cellular and hyperplastic and showed almost complete failure of maturation, the predominating cell being primitive in type. The third, or moderately mature group, showed cellular marrows in which the degree of failure of cell maturation was less than in the second group. The fourth group was one in which the marrow had been replaced by the tissue of Hodgkin's granuloma.

Supravital studies by the method of Sabin were made on all the biopsy material. These differential counts corresponded quite well with the appearance of the marrow as seen in sections. The similarity of the cell types present in the failure of maturation occurring in acute agranulocytosis to those occurring in the marrows of cases of aplastic anemia is a striking and important fact.

MYELOSARCOMATOSIS. Theodore R. Waugh, Montreal, Canada.

Abstract. The term myelosarcomatosis has been employed by various authors to designate atypical systemic myeloid processes. A review of these cases shows that they vary all the way from leukemic myeloses with tumor-like nodules to multiple myelomas with rather aggressive manner of growth. If any uniformity of nomenclature is to be preserved this term should be used to signify a process on the myeloid side, which is analogous to the relatively common lymphosarcomatosis on the lymphoid side. That such processes apparently occur is exemplified by the following case.

A male, aged 48, gave a clinical picture suggestive of Kahler's disease. There was marked Bence-Jones proteinuria and rarefaction of the bones. The blood

findings were essentially normal. Death resulted from pulmonary thrombosis. Autopsy revealed multiple nodules in the bone marrow with intervening fatty myeloid tissue. These nodules had thinned and expanded the cortex of the bones. There were no tumor masses in the internal organs but the spleen was moderately enlarged.

Histological examination of the nodules in the bone marrow showed areas of pleomorphic myeloid hyperplasia with comparatively few myeloblastic forms, and from these by gradual increase in the number of immature elements a transition into a typically neoplastic appearing type of proliferation. In some areas the dedifferentiation had reached such a degree that the cells presented the appearance of reticular elements. The splenic pulp was diffusely involved by similar immature cells and foci were present between the liver columns. The lymph glands apparently escaped.

It would appear, therefore, that there occurred primarily multiple focal areas of hyperplasia in the bone marrow with transformation to a neoplastic type of proliferation, and secondly, a systemic spread of this immature proliferative process to the spleen and liver. This type of reaction is distinctly analogous to that met with in many cases of lymphosarcomatosis, and hence would appear to justify the name myelosarcomatosis.

ANATOMICAL STUDIES ON PRIMARY AND POSTPRIMARY TUBERCULOSIS IN WHITE CHILDREN AND ADULTS. Kornel L. Terplan, Buffalo, N. Y.

Abstract. The anatomical incidence of tuberculosis among 312 white children between 1 month and 6 years of age was 4 per cent. Among 52 children and young adults ranging from 7 to 18 years of age it was about 20 per cent. In the first group 1 case showed one typical calcified Ghon focus in the lung parenchyma, first detected by postmortem radiographic examination, without complex formation. Similarly, one completely calcified small focal lesion was found in the 2nd case without changes in regional lymph nodes. (Histological serial sections were cut through all lymph nodes draining the sites of the calcified Ghon foci.)

Among 26 white adults, aged 20 to 40 years, 11 showed no signs of tuberculosis in lungs or intestines. (Postmortem roentgen films were taken and thorough macroscopic serial sections cut through both lungs and bronchomediastinal and mesenteric lymph nodes.)

Among 94 cases between 41 and 80 years of age only 4 were entirely free of tuberculous changes. In 7 cases (ages 38, 46, 55, 58, 58, 66 and 81 years) the anatomical findings pointed to a relatively late primary complex with still cheesy or cheesy fibrous changes. Although the primary foci were distinctly encapsulated calcification had not yet taken place. This feature was especially striking in a senile male of 82 years where the cheesy fibrous complex change was limited to two regional lymph nodes. With the exception of 1 case among these seven individuals, in which recent bronchogenic foci were present, spread from the primary focus or from the caseous lymph nodes to more centripetal lymph nodes or into the blood stream was not demonstrated. In 11 cases of the aged group under discussion distinct signs of an old primary focus or primary complex in a decidedly healed ossified or calcified ossified stage were observed, but in addition there were noted relatively recent cheesy, cheesy fibrous, or cheesy chalky fibrous, foci and complex changes in their regional lymph nodes which could not be demonstrably connected with the healed primary foci or

complexes. These last changes occurred in different areas of the lungs with separate lymphatic drainage; only in 1 case were they present in the same lobe as the healed lesion.

Conclusions: According to the postmortem figures at the Buffalo General and Children's Hospitals obtained from a systematic study over a period of 3 years primary tuberculous infection does not necessarily occur only in childhood. It is realized that the number of cases examined is too small to permit of definite statistical conclusion. Nevertheless, the anatomical results correspond approximately to the incidence of tuberculin-positive children and young adults between 10 and 18 years of age in the schools of Buffalo. The number of tuberculin-positive reactors lies between 20 and 25 per cent. From this study it seems likely that the primary focus or primary complex may be acquired very often in the years following puberty, *i.e.*, from the latter part of the second to the third and fourth decades. Evidence is at hand to warrant the belief that infections acquired after childhood may restrict themselves to focal and complex changes, as known in children, without producing active tuberculosis as a disease. In cases with anatomically healed Ghon foci or primary complexes, a second tuberculous infection may occur and produce again a so-called tuberculous complex with a focal lesion with involvement of regional lymph nodes. These second complexes point to true exogenous reinfection. Compared with the first healed complex they are by all available anatomical evidence relatively recent and decidedly younger.

Discussion

(Dr. Esmond R. Long, Philadelphia.) Dr. Terplan's results furnish interesting anatomical correlations with our epidemiological work. Twenty-five years ago in a city the size of Buffalo it would have been extraordinary to find an incidence of infection at the age of 40 as low as that given, with considerable increase later in life. He reported 60 per cent at that age, where formerly 95 per cent might have been expected. That probably represents the present situation throughout our own country. As a result of the antituberculosis campaign we are postponing the age of onset of primary tuberculosis. Our tuberculin tests at the present time show some such incidence as Dr. Terplan has indicated, but prior to this time we have not had good anatomical evidence that primary infections occur this late in life and pursue just the same course as they do when they occur in childhood. I think these results effectually dispose of that old belief that adults who escape primary lesions when they are children, such as people from the mountains and remote country districts, who come to the city late in life, are likely to break down with a massive primary tuberculosis. The actual fact is that if they develop primary tuberculosis it tends to pursue the same benign course as though they had developed it at the age of 5 or 6 years.

(Dr. Herbert S. Reichle, Cleveland.) The 8 cases of primary infection in the older age group are particularly interesting, but in addition to the entirely plausible view that these represent the first and only primary tuberculous infections in these individuals, there is just one other possibility which I think must be considered. I do not believe that the suggestion to be made is true in all cases; some, however, might be interpreted in this particular fashion. Huebschmann, who has been interested in tuberculosis for some years, pointed out that many primary infections have a central core of granulation tissue which

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usually escapes our observation because we take but one section through the focus. Along the path of this granulation tissue true absorption may occur, not only of the calcium, but also of the bone, and thus a true primary focus may disappear entirely. I think he has proved his point in so far as it concerns the lung, but I do not know whether the same process is possible in the lymph nodes. It might be that in such cases the primary focus of childhood had already disappeared at a time that the second primary infection developed, which latter phenomenon has been discussed by Dr. Terplan.

(Dr. Terplan, closing.) I am, of course, familiar with Dr. Huebschmann's work on tuberculosis. However, I do not know whether he actually has proved the complete resorption of a primary Ghon focus in a child or young adult. Huebschmann believes that the first tuberculous lesion in the parenchyma of the lung is originally a pure exudative lesion of pneumonic nature, and he considers the possibility that even in this stage a resorption of the exudate may take place before the focus becomes caseous. In his opinion not even a small scar may remain. In my opinion this certainly would be, if at all possible, very exceptional from all that we know about primary tuberculous foci in man. I do not, however, question that small ossified lesions finally may be resorbed completely.

I saw in very rare cases and only in senile adults minute fragments of bone tissue in the lungs practically without any reaction in the nature of scar tissue surrounding them. One point I should have stressed a little more. In examining lungs from children and young adults for Ghon foci it was customary first to look for tuberculous changes in lymph nodes, and then to search for the focal lesions in those areas that were drained by the tuberculous lymph nodes. When the lymph nodes appeared normal it usually was taken for granted that there was no Ghon focus present, and these cases were considered negative. In 2 cases in my series, however, there were typical calcified Ghon foci, one of which was detected only by X-ray, and, still, complete serial sections through the lymph nodes regional to the Ghon foci did not reveal any tuberculosis.

It is therefore absolutely necessary to make routine roentgen photographs of lungs, especially in children and young adults, and, in addition, complete gross dissection of all lobes, before we feel sure that a case may be called negative.

THE HEALING OF TUBERCULOUS CAVITIES. A STUDY BY SERIAL SECTIONS. Esmond R. Long and (by invitation) Gertrude Duetz, Philadelphia, Pa.

Abstract. In an autopsy on a patient dying from chronic ulcerative and fibroplastic pulmonary tuberculosis with extensive cavitation of the right lung a cavity was found in the left lower lobe which appeared to be healing. This cavity was 4 cm. in diameter and approximately spherical. Its outlet appeared to be extremely narrow and was pointed directly cranially. No fresh bronchogenic tuberculosis was found which could be related to dissemination of infectious material from this cavity, although the cavity was filled with a white semiliquid material containing numerous acid-fast bacilli. It appeared evident from the smallness of the outlet and the lack of surrounding infection that the cavity was not draining.

Serial sections were made of the neck of the cavity and the adjacent pulmonary tissue. In these sections several regions of healed, in some cases calcifying, bronchiogenic tuberculosis were found. One of these was connected with the cavity by a heavy band of scar tissue, in which traces of a lumen remained,

filled with degenerated cellular exudate. The calcifying mass at the distal end appeared to represent the inspissated contents of a cavity that had lost its outlet by scar tissue obliteration.

The outlet of the main cavity appeared to be undergoing a similar fate. It was lined by thick granulation tissue and filled with degenerating leukocytes. The diameter of the outlet at the cranial end was approximately 1 mm. The contents were so dense as to suggest that no movement leading to drainage could take place.

The entire system of healing tuberculous lesions appeared to have been due originally to bronchiogenic dissemination from the opposite lung. The caudiocranial direction of the main and subsidiary outlets apparently slowed up drainage, after cavitation occurred, giving time for fibrosis of the outlets and further limitation of drainage. The study emphasizes impeding of drainage as the factor of chief importance in the healing of cavities. This factor is of clinical importance, not only in the common spontaneous healing of small cavities, but also in induced healing following artificial lung collapse. In the procedure the closure of soft-walled outlets of small cavities is probably of as much importance as pressure collapse of large cavities.

TUBERCULOSIS OF THE MAJOR BRONCHI. H. S. Reichle and (by invitation) T. T. Frost, Cleveland, Ohio.

Abstract. Tuberculosis of the major bronchi can be classified according to pathogenesis as (1) infection by implantation, (2) infection by contiguity, and (3) infection by continuity. The first is decidedly less common than the other two, a fact that may be ascribed to the protective influences of cilia, mucous and bronchial peristalsis. Infection by contiguity is prone to occur because of the proximity of the extracartilaginous mucous glands to diseased lymphatics and lymph nodes. Proliferation of the adventitia and atrophy of the mucous glands tend to close this avenue. Infection by continuity occurs secondarily to an implantation tuberculosis in the lower lobes and in the bronchi draining a tuberculous cavity. Major bronchi appear singularly resistant to tuberculosis; however, when fibrosis has transformed them into rigid tubes and mucosa and submucosa are destroyed and replaced by tuberculous granulation tissue, an open focus of disease is created which is rarely closed either by natural processes of healing or by pneumothorax or thoracoplasty.

Discussion

(Dr. Kornel Terplan, Buffalo.) We had occasion to see a case similar to Dr. Reichle's interesting observations in a completely healed stage. This case was that of a woman about 35 years of age with a known history of chronic tuberculosis many years previous to death. The clinical diagnosis was dextrocardia. She died suddenly from lobar pneumonia of the left lung. We found at postmortem complete fibrous obliteration of the main bronchus affecting the entire lumen about 1 to 2 cm. distal to the carina. The surrounding tracheobronchial lymph nodes showed complete calcification. The obliteration of the main bronchus had, of course, produced complete collapse of the right lung with consequent shift of the mediastinum to the right and "dextrocardia."

POSTOPERATIVE LYCOPODIUM GRANULOMAS. I. H. Erb, Toronto, Canada.

Abstract. Six cases are reported in which there developed postoperatively a granulomatous lesion, the result of the introduction into the field of operation of numbers of lycopodium spores. For some years these spores have been used in many operating rooms as an ingredient of dusting powder for rubber gloves.

The lesion produced is of the nature of a foreign body granuloma and bears a striking resemblance to tuberculosis, with which it may be easily confused. As the spores are acid-fast, they present a striking picture in sections stained by the Ziehl-Neelsen method, which procedure is of value in differentiating the condition from tuberculosis.

The lesion is of importance, not only because it may result in intestinal obstruction through the formation of adhesions, but also because of the fact that, if unrecognized, it may lead to errors of diagnosis and thus to unnecessary surgical or other therapeutic measures.

It is the opinion of the author that the use of lycopodium spores as a dusting powder in operating rooms should be discontinued.

Discussion

(Dr. Ralph D. Lillie, Washington.) Shortly after the appearance of the article last fall we wanted some granulation tissue in large quantities so we thought we would try lycopodium in rats, but we made the mistake of sterilizing the lycopodium spores in a suspension of salt solution in the autoclave, and when we injected them into rats we did not get the tumors we had been led to expect. We made sections where they had been put in and could not find anything except a little yellow material. There was no particular granulation tissue reaction and the lycopodium spores which were there were no longer acid-fast. Just about that time I came down with influenza and went to bed, and when I got up I found too much encephalitis going on to follow it up. It seems to me, however, we must have hydrolyzed whatever it is that causes this foreign body reaction by autoclaving wet and under pressure.

(Dr. David A. Wood, San Francisco.) About 2 years ago I had the opportunity of seeing one of these lycopodium granulomas from a man 27 years of age who had had a laparotomy 3 months before. In routine stains the lycopodium spores were refractile. The people in the West are looking for various mycotic infections, and it is a good point to keep in mind that one should look at lycopodium granulomas carefully, because occasionally some spores are oval and, in the routine stains, one might easily mistake them for the empty spores of *Coccidioides immitis*. In my particular case the giant cells were very beautiful with many of the lycopodium spores inside. So the matter of mycotic infections is just another point to be kept in mind in the differential diagnosis.

(Dr. Erb.) In reply to what Dr. Lillie said, we did not try sterilization of any of these spores in saline or other fluid. Of course in the operating room they are always sterilized dry, but under pressure. We did, however, inoculate some guinea pigs, and we thought we were going to get beautiful lesions but we were disappointed. I thought we would get little nodules all over the peritoneum, but the spores were all picked up in the lymph nodes where they were surrounded by foreign body giant cells. We did not find the typical concentric arrangement.

(Dr. Howard T. Karsner, Cleveland.) In an attempt to produce pulmonary hypertension we have injected lycopodium spores intravenously into dogs. The

animals have survived for more than a year. The presence of the spores in the arterioles and capillaries is accompanied by the development of small granulomatous masses but without multinucleated giant cells, perhaps because the spores are within the vessels.

GRANULOMATOUS MYOCARDITIS. A CASE FOR DIAGNOSIS. James Miller, Kingston, Ontario.

Abstract. A series of cases of myocarditis of a subacute type and of doubtful origin has been reported in the literature from time to time. It is uncertain if these can be regarded as being due to one and the same causal germ. Most have been thought to be tuberculous, but complete proof has usually been wanting. The following case seems to fall into the category of subacute myocarditis of bacterial origin due to some unknown virus.

A Chinese, after showing a series of metastatic inflammatory foci in different parts of the body during a period of some months, died of acute peritonitis, pericarditis and myocarditis. The heart showed a diffuse myocarditis with extensive areas of necrosis and hemorrhage in the wall of the left ventricle. Microscopically the condition was definitely inflammatory and the cells were predominantly mononuclear. No organisms could be found either microscopically or on culture, and there were no spirochetes or tubercle bacilli. There was a single ulcer in the small bowel, transverse in direction, with raised margins. It seems probable that this may have been the origin of the peritonitis. The pericarditis was accounted for by the underlying inflammation of the heart muscle. There was no lesion of the coronary artery.

This case falls into the category of subacute myocarditis on account of the predominance of mononuclear cells in the areas of infiltration. There was no histological support for the view that it was tuberculous, apart from the extensive necrosis. There was scarring at the apex of the left lung and there was one ulcer of the small bowel, grossly suggestive of a tuberculous lesion, but the microscope revealed the same type of cellular change as in the heart muscle. One metastatic focus of infection was examined during the life of the patient but it also showed changes of a non-specific type.

In view of the absence of proof of the nature of the causal germ the term "granulomatous myocarditis" is suggested as covering best the histological and gross characteristics of the case.

Discussion

(Dr. Howard T. Karsner, Cleveland.) Problems such as presented by this case are of profound interest. Having seen the specimen and heard the presentation several questions arise. It is not necessary to exclude syphilis as a cause by virtue of the negative Wassermann test and the inability to demonstrate spirochetes. There are, however, no clearly positive stigmata of syphilis. The number of instances of isolated acute myocarditis of Fiedler is small and nothing is known of those cases which may perhaps progress to healing. The extensive necrosis in Dr. Miller's case is not duplicated in isolated acute myocarditis and in this latter condition hypertrophy appears to be constant. The condition in this heart might possibly be attributed to coronary disease, for although the larger coronaries were normal, it is still possible that examination of the minute twigs of the coronaries might show significant disease in that situation. I have

observed multiple small areas of fibrosis in the myocardium, by no means identical with the granulomatous lesion of this case, in hearts without gross coronary disease but with extensive sclerosis of small twigs.

(Dr. Ralph D. Lillie, Washington.) Something about this case recalled to my mind the lesions we have been studying recently in experimental subacute tularemia in animals. There is in that disease, not infrequently, a granulomatous myocarditis with peritoneal involvement which may be granulomatous. I offer this merely as a possibility. We do not know all about human tularemia yet.

(Dr. Miller.) I agree that a negative Wassermann does not exclude syphilis. I am not familiar with the work of Fiedler and I hope Dr. Karsner will give me the reference.

As to what Dr. Lillie said about tularemia, that did enter my head as a possibility, but with a history such as this, lasting 8 months, it seemed highly unlikely.

(Dr. Lillie.) It is not usual, but there have been a few cases which have gone on for 1 or 2, or even more, years.

(Dr. Miller, closing.) That is a matter which one might take further into consideration.

RADIAL INCLUSIONS OF GIANT CELLS. Edwin F. Hirsch, Chicago, Ill.

Abstract. Giant cells with rosette inclusions have been observed in lesions simulating tuberculous or foreign body granulation tissues. The nature of these inclusions has been an enigma and many divergent opinions have been recorded regarding their composition and the significance of the associated lesions. My observance of these granulation tissues has been in the lungs, spleen or lymph nodes of 8 bodies postmortem, and in 26 tissues removed surgically. The most conspicuous lesions observed in the postmortem material were in the spleen, lungs and parabronchial lymph nodes of a man, aged 72 years, who for many years had had urinary concretions, pyelitis and cystitis. He had taken one ounce doses of olive oil, presumably for some time. His spleen weighed 290 gm. and had many discrete fibrous nodules several mm. to 2 cm. in diameter. By chemical extraction the spleen contained quantities of fat melting at 65° C to 75° C and, when cool, containing rosettes like the inclusions and the crystals of palmitin or stearin.

All authors state that the radial inclusions in the giant cells are colored by nuclear and elastin stains, especially by the purple of Mallory's phosphotungstic acid hematoxylin stain. Wolbach and others have described tinctorial differences indicating that the inclusion material may be a mixture. The inclusions do not react with fat stains. Their configuration is crystalline and therefore they form in tissues according to the usual laws governing crystallization. A crystalloid in an aqueous system, according to Schade, separates in concentrically laminated spheres in the presence of small amounts of colloidally dispersed protein. Theoretically, at least, the deduction follows that the radial inclusions of the giant cells separated from a solvent not aqueous.

Lipins stimulate foreign body tissue reactions, notably cholesterol. Intravenous injections of palmitin and stearin in olein and of soaps of calcium or magnesium in olein failed to produce the characteristic lesions with giant cells. Mixtures of stearin and palmitin and cholesterol in olein injected intravenously into rabbits produced lesions in the lungs comparable to those observed

in human tissues, except that the crystalline fat inclusions were not completely reproduced. That is, only the peripheral portions, the spines, of the crystalline fat had become insoluble. Such portions stained according to the descriptions given for the radial inclusion.

These experiments, of course, indicate, simply, that a lipin mixture containing cholesterol may produce tubercle-like lesions containing giant cells and that crystals of palmitin and stearin or their derivatives may become insoluble rosette inclusions of the giant cells.

ETIOLOGY OF CONGENITAL BILATERAL POLYCYSTIC KIDNEYS. James E. Davis, Detroit, Mich.

Abstract. Illustrations of the evidences of tissue deficiencies are apparent in the quantity and quality of the structures of the congenital bilateral polycystic kidneys at all ages. The stroma in these kidneys, particularly that in proximity with the immature epithelial parts, is of mesothelial character. This observation can be confirmed in kidneys from patients over 50 years of age. The quantity of epithelial tissue present in both kidneys is markedly deficient. Part of this deficiency may be inherited and part acquired through the influence of cystic degenerative changes. The inherent growth impulse of the epithelial tissue is inadequate and unbalanced. There are numerous contrasts of unassembled, partly assembled and completely assembled epithelial cells. The assembled cells exhibit the characteristic diffusely appearing cystic degenerative change. The arrangement of the unit parts of this type of kidney does not enable a conclusive opinion whether there is here strictly a metanephric condition of defectiveness or not.

Discussion

(Dr. Kornel Terplan, Buffalo.) I should like to ask Dr. Davis whether he has observed any hyperplastic changes in small arteries and arterioles in those polycystic kidneys in stillborn and very young infants in which practically no normal parenchyma had remained. I saw such changes in markedly hypoplastic kidneys in young infants.

(Dr. Davis, closing.) I have not observed any hyperplastic changes in the arteries.

DISAPPEARANCE OF GLOMERULI IN CHRONIC KIDNEY DISEASE. Alan R. Moritz and (by invitation) J. M. Hayman, Jr., Cleveland.

Abstract. The total number of patent glomeruli in a series of normal and abnormal kidneys was determined by Kunkel's modification of Vimtrup's method. Before the kidneys were macerated for counting the number of injected glomeruli blocks were taken for histological examination and the ratio of injected, uninjected and obliterated glomeruli was determined. From this ratio and the total number of injected glomeruli the total number of recognizable glomerular structures was calculated. This total included all scars that might possibly represent glomeruli. It was found that in arteriolar nephrosclerosis, chronic diffuse glomerulonephritis and chronic pyelonephritis a large proportion of the glomeruli may disappear completely.

The mode of absorption of the glomerular scars was studied in uninterrupted series of sections. It was found that the hyalinized spherical scar of the obliter-

ated glomerulus is penetrated by argentophilic fibrils which eventually become continuous with and conform to the pattern of the interstitial reticulum of the kidney. The hyalin undergoes peripheral and central rarefaction and disappears.

Experimental glomerular damage was produced by unilateral X-ray exposure of temporarily exteriorized kidneys of rabbits. Complete disappearance of a large proportion of glomeruli was observed following such treatment.

It was concluded that since glomerular scars disappear completely less information as to the severity and type of renal injury may be obtained from the microscopic examination of kidneys from cases of chronic Bright's disease than is commonly supposed.

THE MORPHOLOGY OF THE SENILE PROSTATE. Robert A. Moore, New York City.

Abstract. The morphology of the normal prostate varies with the age of the individual. In senility, as contrasted with young adulthood, there is a decrease in the height of the epithelium, a decrease in the size of the glands and a loss of the papillary infoldings. In association with these epithelial changes there is a fibrosis of the stroma, both intra- and interlobular, and a corresponding decrease in smooth muscle fibers. By comparison with the experimental effects of gonadectomy it would seem probable that these alterations are related to a decrease in the male sex hormone output of the testes. If there be an associated arteriolar sclerosis the acini undergo a peculiar type of complete atrophy.

Discussion

(Dr. Alan R. Moritz, Cleveland.) I should like to know if the arteriolar change is part of a systemic arteriolar sclerosis or if it is an isolated lesion, as seen in the senile uterus and ovary.

(Dr. Moore.) This series of prostates was taken from Dr. Erdheim's service, where there were no microscopic examinations, but, in the few cases in which there was a record in the autopsy protocol of nephrosclerosis, this type of process occurred in the prostate. It is my own belief from the few cases where I have complete material at Western Reserve and Cornell that this change is a part of a generalized arteriolar disease and is not a functional process, such as occurs in the uterus and ovary.

THE ISSUES AT STAKE IN THE GRADING OF TUMORS. Stanley P. Reimann and (by invitation) Clark E. Brown, Philadelphia, Pa.

Abstract. Two hundred cases of carcinoma of the breast have been graded histologically and their subsequent histories determined. The biological principles which are either consciously or unconsciously evoked when attempts at tumor grading are made are discussed, and the conclusion is drawn that cellular growth is too dynamic a process and the variables are too numerous for a static bit of evidence, like histological slides, to be of use in the prognosis of any individual case.

Discussion

(Dr. William Carpenter MacCarty, Rochester, Minn.) I should like to make three points in connection with this subject. First, I want to commend the speaker on his conservatism, with which I thoroughly agree.

In the second place, I think probably I am one of the most active individuals who have studied this problem. I am one of the few individuals who have written much about it. In my opinion grading has only biological significance. It is of interest to those of us who are interested in the behavior of cancer, and I may say that is my particular interest in the subject.

The third point is the lack of specificity in the percentages. Suppose I said to our President here: "You have a Grade 4 cancer," which I hope he has not. I am the pathologist talking to the surgeon. He will say to me: "Dr. MacCarty, what is the prognosis?" and I will say, "I will give you the percentages." Suppose I tell him that in Grade 4 cancer in 80 per cent of the cases death takes place in say 2 years, and that in 20 per cent they live longer than that, 5 years, let us say. My friend will then say: "You tell me I have a Grade 4 cancer, and you also say that 80 per cent live 2 years, and 20 per cent live 5 years, or longer, but I am particularly interested in myself — how long am I going to live? That is what I want to know." Then I will have to fold up my wings and drop my tail and say, "I am awfully sorry; I am a scientist but I cannot tell you that." If I were a surgeon, and I presume I have seen more surgery than any surgeon, there would be one main criterion by which I would decide to remove a tumor, benign or malignant. I would first decide whether the patient was in good condition — heart, kidneys, tissue fluids, age and so forth. I would then decide whether there was any glandular involvement which I could not reach, and after I had decided I could remove the tumor and glands I would do so, and I would not care what grade it was. I have seen patients with Grade 4 tumors, the worst grade, live many years, even in spite of the fact that they have had glandular involvement, simply because the growth was easily accessible and the surgeon did a radical job. I want to emphasize that I think this grading has biological interest more than it has practical importance, so far as the individual is concerned.

(Dr. Shields Warren, Boston.) I have been interested in grading, not so long a time as Dr. MacCarty has, but I have profited very largely by his example and advice, and have also profited extensively by Dr. Reimann's work in the field of the breast.

I wish to emphasize one point that was made by Dr. Reimann and Dr. Brown — that the environment is a very important factor. In the study of the material that I presented yesterday it was noticeable in relation to age, known to be an important factor in determining clinical malignancy (that is, young people in general doing more poorly with a given tumor of a given extent than older people), that there was no difference in the grade of the tumors in a young person as against those in an older one. The percentage ran about the same. The other point which I think all pathologists who have to deal with surgeons have run up against is the temptation in the lower grades for the surgeon to attempt a less radical operative procedure. I feel that, if we do grade tumors, we must also constantly remind those who depend on our diagnosis that cancer is cancer, whether it is Grade 1 or Grade 4.

(Dr. Brown, closing.) I should like to thank Dr. MacCarty and Dr. Warren for this constructive criticism, and I am sure we are in hearty accord with everything they have said. I might also bring to mind in closing, drawing from the rich elocutionary achievements of last night's dinner performance, that contention as well as hope springs eternal in the human breast. Hope certainly is needed in dealing with the biological fundamentals of cancer.

MELANOBLASTS OF THE ANAL CANAL. THEIR RELATION TO PRIMARY MELANOMA OF THE RECTUM. George F. Laidlaw and (by invitation) Charles L. Janssen and A. Purdy Stout, New York City.

Abstract. The anal canal is lined with stratified epithelium of ectodermal origin. This epithelium contains abundant melanin and melanoblasts up to the mucocutaneous line. Above this line there are no melanoblasts and no melanin. Here the mucosa consists of tubular glands of endodermal origin. It is probable that primary melanoma of the rectum arises from the melanoblasts of the anal canal.

Discussion

(Dr. Shields Warren, Boston.) I should like to ask Dr. Laidlaw what he regards to be the mode of production of the submucous pigmentation in the so-called melanosis coli.

(Dr. B. Earl Clarke, Providence.) A year ago I reported before this Association a tumor which, as nearly as we could determine, was primary in the upper jejunum. At that time I found in the literature 1 other case of primary melanoma of the jejunum reported by Dr. Lund of Boston. Has Dr. Laidlaw made any study of melanin-producing cells in the small intestine, and what is his opinion as to the mode of origin of these tumors?

(Dr. Ernest M. Hall, Los Angeles.) Two cases of supposed primary melanoma of the small intestine were discussed at a recent meeting of the Los Angeles Pathological Society. One of these tumors, reported from the Los Angeles General Hospital, was the size of a walnut; the other, reported by Dr. Foord from the Pasadena Hospital, was the size of a man's fist. There were two tumors present, in the latter case, of almost equal size and no melanomas could be found elsewhere.

(Dr. Victor C. Jacobsen, Albany.) I have been studying melanin for many years and trying to see if we can explain melanin, wherever it is found in the human, as originating chiefly in the neuro-ectodermal cells. Almost all of it can be so explained. There are a few exceptions and Dr. Laidlaw has mentioned them. One condition that has given difficulty is melanosis coli. The colon in extreme cases of constipation or of chronic obstruction low down may become intensely pigmented with true melanin. The melanin is present in cells in the mucosa beneath an intact epithelial lining. These cells are "dopa"-negative. Hence they do not make melanin; they simply phagocyte it. The question is, where does it come from? In studying the excretion of melanin in the body the kidney has been proved to be an important source of excretion, and of course the skin by desquamation. A certain amount must escape into the intestine along with foodstuffs of animal and vegetable nature which contain melanin. It is quite apparent that in the intestine are all the precursor substances necessary to form that molecule of unknown composition which is melanin. It is very probable that there is an actual synthesis of melanin in the intestine, particularly in constipated people, and that its presence in melanosis coli merely means that it is being phagocyted and not actually made by the cells which contain it.

(Dr. Ralph D. Lillie, Washington.) In regard to melanosis of the colon in minor degrees, it is quite frequent, according to Henschen and Bergstrand. We did some work with it in the appendix in Washington. It appears to me that melanin is finally formed in the large round phagocytes immediately beneath the epithelium, because in quite a number of cases we have seen granular material

not staining like true melanin and occurring first as fine unpigmented granules, then as finely granular brown pigment in the large round cells just under the epithelium.

(Dr. Laidlaw.) Beginning with the last question, Dr. Lillie says "staining like true melanin." What is the criterion for staining like true melanin?

(Dr. Lillie.) The melanin which is stained in melanomas takes an olive green color with toluidin blue, and we find all gradings from a very faint blue color into a dirty olive green in the cells immediately under the epithelium in melanosis of the appendix.

(Dr. Laidlaw.) In forming conclusions from staining reactions we should bear in mind that the thing we call melanin does not always stain uniformly. Dr. Lillie's observation of the behavior of melanin in the appendix is paralleled in silver staining of pigmented skin. If a section of such skin be dipped in silver the melanin in the epidermis will turn black in 5 seconds, but the melanin in the derma will show all grades of staining from black to no staining at all. After several hours or possibly several days in silver all of the melanin granules in the derma will be black. Nevertheless, we call all of these granules melanin, whether they stain slowly or rapidly. It is our experience that intestinal melanin is particularly difficult to stain, sometimes requiring hot silver and many hours to secure uniform staining. It seems to me that Dr. Lillie's brown granules in the appendiceal mucosa are ordinary intestinal melanin giving the customary phenomenon of different degrees of color reaction.

(Dr. Lillie.) We relied on the insolubility in mineral acids, the tinging with basic anilin dyes and the negative iron reaction, making three criteria, and used silver in relatively few cases.

(Dr. Laidlaw.) In regard to melanosis coli, the first question is this — Was the melanin manufactured on the spot by the cell which contains it, or was it merely phagocyted? The best answer to this question is a positive or negative "dopa" reaction. An important precaution should be noted here. A "dopa" reaction must be carried out on fresh tissue. Autopsy material secured many hours after death is unsuitable and uniformly gives a negative reaction. We have been fortunate in securing fresh specimens of melanosis recti and one of melanosis coli from surgical operations. There was abundant melanin in the mucosa but the "dopa" reactions were uniformly negative. We conclude, therefore, that the melanin was not made by the cell which contains it but that it was phagocyted. If phagocyted, where did the melanin come from? The most likely source is the contents of the intestinal canal. Melanin occurs in so many foodstuffs, both animal and vegetable, that it may easily be present in the food refuse of the intestinal canal. The association of melanosis coli with chronic constipation and obstruction would seem to be explained by prolonged contact permitting better absorption of the melanin. I admit freely that these simple mechanical explanations leave something to be desired. The question of melanosis coli, like that of most melanoses in the human body, has not received its final explanation.

Returning to the questions of Dr. Clarke and Dr. Hall, the supposed occurrence of primary melanoma in the upper intestine where normally no melanoblasts have been found presents a problem for which I do not yet know the answer. Possibly the answer is that of Oberndorfer that such tumors are really secondary to a primary tumor which has been overlooked. As a working hypothesis, in our laboratory we believe that in all probability primary melanoma appears only where melanoblasts already exist.

A MALIGNANT HEMANGIOMA WITH METASTASES. E. M. Hall, Los Angeles, Cal.

Abstract. The metastasizing hemangiomas are not only rare tumors but are also interesting because of their relation to the general malignancy problem. All autopsied cases should, therefore, be recorded in the literature.

In the case to be reported the patient was a well developed, white female, married, and 40 years of age. She had felt weak and exhausted for a period of 3 months. She became markedly anemic (hemoglobin 30 per cent) and dyspneic, with rapid pulse and low blood pressure. The presence of a cervical polyp suggested uterine bleeding as the cause of her anemia. She developed a massive right-sided hemothorax and died.

The autopsy, performed 3½ hours after death, revealed a well developed white female with marked pallor of the skin and mucous membranes. There were no angiomatous or pigmented moles anywhere in the skin.

The right pleural cavity contained about 3000 cc. of bloody fluid, while the left pleura contained nearly a liter of blood-stained fluid. The pericardium and heart were normal except for an abnormality of the right coronary artery which showed a small dimple in the aorta at the usual site of origin, opposite the right cusp, but no opening into the vessel. The coronary artery was present, but only about one-half normal size.

The right lung was almost completely collapsed and about the size of a man's fist. A number of small, dark, blood-filled tumors were present in the pleura, in the lower portion of the upper lobe and throughout the lower lobes. Along the lower border of the lower lobe there was a firm reddish mass 5 by 2 by 1.5 cm., with a roughened surface. This mass was composed of angiomatous tumor surrounded by hemorrhage, and was likewise the source of the bloody pleural effusion. The left lung was only partially collapsed. There were about twenty similar angiomas in the pleural surfaces, with two larger nodules present, the largest measuring 2 by 1.5 by 1.5 cm. A few angiomatous areas were found deep in the lung parenchyma, but most of them were subpleural.

The spleen, adrenals and kidneys were normal. The liver contained a number of mottled, reddish gray tumor nodules 1 to 1.5 cm. in diameter. These had the appearance of vascular carcinomatous metastases. The largest of these was on the under surface of the right lobe, measured 2.5 cm. in diameter, and was somewhat raised above the surface of the liver.

A number of moderately swollen hemorrhagic lymph nodes bordered the pancreas and a firm mass the size of a man's fist was found immediately below the pancreas in the midline. On cutting through this mass it was found to consist of fibrofatty tissue surrounding a group of 13 to 15 enlarged hemorrhagic lymph nodes. Similar nodes were found along the aorta as far as the bifurcation.

The aorta appeared somewhat smaller than normal. The leptomeninges and the brain substance were very pale. The vessels at the base of the brain were considerably smaller than normal. The bone marrow of the sternum was pale red.

Histological examination of one of the tumor nodules (lymph node) shows one part consisting of large, thin-walled spaces filled with red blood cells and lined by flattened endothelium. The other portion of the gland is cellular, consisting of small, irregular or collapsed spaces lined by large atypical cells varying from fusiform to large polygonal. In the latter the nuclei are oval or spherical, many are hyperchromatic and occasional mitoses are seen. The cellular portion has a distinctly malignant appearance.

The sections of the lung show quite a similar picture, except that the cavernous spaces are partly confluent and in several places thrombosed, with more or less hemorrhage into the surrounding tissue. One of the sections from the liver shows a fairly large vein, the wall of which is invaded by the tumor with irregular masses of tumor cells within the lumen.

The primary tumor was probably in the right lung, although one cannot say with any great degree of certainty. The tumors of the liver were decidedly like metastases in appearance. It is evident that the tumor was histologically malignant, as well as producing widespread metastases. Evidence of congenital vascular deficiency is shown in the presence of an imperforate right coronary and in hypoplasia of this vessel, together with the cerebral vessels and the aorta.

Discussion

(Dr. Kornel E. Terplan, Buffalo.) I should like to ask Dr. Hall whether the lymph nodes draining the lungs were involved. If the bronchomediastinal chain was not involved it may be well to consider whether the primary site of the tumor was not in the retroperitoneal lymph nodes. I happened to see in Prague 2 cases of malignant hemangioblastoma which apparently originated in the retroperitoneal lymph nodes, producing extensive metastases in the liver. One of these cases had also metastasized to the lungs.

(Dr. Hall, closing.) I am very much interested to hear what Dr. Terplan has to say. The question in my mind was this — whether the tumor was primary in the retroperitoneal lymph nodes or in the right lung. The lymph nodes in the mediastinal chain were not involved in this case. Also, I might add that the pain in the right lumbar region and in the right hip could be accounted for by the tumor mass below the pancreas.

SUPERIOR PULMONARY SULCUS TUMOR (PANCOAST). B. Earl Clarke, Providence, R. I.

Abstract. Pancoast in 1932 reported 7 cases which he believed to represent a new disease entity and for which he proposed the name of "superior pulmonary sulcus tumor." He predicates such a diagnosis upon the following findings: (1) pain around the shoulder and down the arm; (2) loss of power and wasting of the muscles of the hand; (3) the presence of a Horner's syndrome; and (4) a characteristic roentgenographic picture of "a comparatively small and circumscribed shadow in the apex, due to lung displacement, together with destruction of the posterior portions of one or more ribs and the adjacent articular and transverse processes and possibly involvement of the bodies of one or more vertebrae."

None of Pancoast's 7 cases came to autopsy. In 2 there was surgical intervention and tissue was obtained for histological examination. The 1st of these, operated upon in 1921, was originally said to be an endothelioma. However, a slide from this case was seen by Dr. Joseph McFarland, and in his opinion the histology is that of a "carcinoma spinocellulare." In a 2nd case, operated upon in 1922, the histological diagnosis was carcinoma. Apparently no slide was available for study. So far as I can learn the postmortem findings in such a case have never been recorded in the literature.

Our patient, a white male, 53 years of age, entered the Rhode Island Hospital Sept. 19, 1933. He presented clinical and X-ray findings in every way typical of those described by Pancoast (lantern slides of roentgenographs shown).

At postmortem examination the apex of the left lung was found adherent to the underlying tumor mass. When pulled away it left a flattened area on the posterior aspect of the apex. Tumor tissue was found to extend into the lung substance only 0.5 cm. beneath this flattened surface.

The tumor extended around the posterior aspect of the upper thoracic cavity destroying the posterior 3 inches of the left third rib and about 1 inch of the left second rib. It extended across the midline destroying the entire body of the second dorsal vertebra and parts of the bodies of the first and third vertebrae. The tumor pressed upon the spinal cord but the dura was not invaded. A small portion of the right second rib was destroyed. The apex of the right lung was not adherent. The nerve roots and the posterior root ganglia were lost in the tumor.

The histology of the tumor is typically that of an epidermoid carcinoma. In the more differentiated portions the cells occur in rather large sheets and have the characteristics and arrangement of prickle cells. In a few places actual protoplasmic bridges are demonstrable. There is no keratinization. Other parts are poorly differentiated, but the cells are thought always to be epithelial in type (photomicrographs shown).

The origin of this tumor is not entirely clear. Three possible sources are to be considered. We include the pleura because the original histological diagnosis of Pancoast's first case was "endothelioma." Pancoast asks why such a growth in this one location should always invade bone and never elsewhere? McFarland rejects the histological diagnosis of endothelioma. Surely my case cannot be of pleural origin. I think we can rule out the pleura.

The possibility of primary carcinoma of the lung cannot be so lightly passed over. We know that tumors of similar histology do originate in the lung. McFarland (quoted by Pancoast) points out that tumors of this cell type are not so apt to be found in an apical lung lesion so far away from the larger bronchi. Again we might ask why, in this one location, should a primary lung tumor always invade bone and never elsewhere? It seems to me highly improbable that a tumor of the lung with such meager lung involvement could be primary to such extensive extrapulmonary extension. In spite of all this I am loath to reject this possibility.

A third possibility suggested by Pancoast is an origin from an embryonic rest, similar to that of branchiogenic carcinoma. He postulates such an origin from the fifth pharyngeal pouch (ultimobranchial body). While such a possibility cannot be denied I see no way of establishing its correctness.

I must confess, then, that I am unable to arrive at a definite decision as to the origin of this peculiar tumor. It is quite possible that no one of the above suggestions is correct. It may even be that this is not a pathological entity. Tumors of different histological types in this particular location might produce the same clinical and roentgenological findings.

Discussion

(Dr. Howard T. Karsner, Cleveland.) At this time I wish to place on record another case in which the X-ray findings were those of superior pulmonary sulcus tumor. The patient died after having symptoms and signs for 4 months. At the autopsy a tumor was found in the right superior pulmonary sulcus, involving the lower part of the neck and the upper part of the right lung. This eroded bones locally and was accompanied by metastases to the right adrenal,

right kidney and intestinal tract. The tumor has not been completely studied microscopically, but at the present time a tentative diagnosis of sympathicoblastoma is presented. The tumor was not in a situation to have arisen from the carotid body and presumably is of sympathetic system origin.

(Dr. Paul G. Weston, Jamestown, N. Y.) Some months ago I found a tumor at autopsy in exactly the same site as that described by Dr. Clarke, but it extended back to the foramen into the spinal canal and had its origin from the spinal dura. Grossly it was a fairly well circumscribed tumor, about the size of one's fist, had displaced the esophagus, as Dr. Karsner's case had, but did not show any metastases anywhere. The histology was that of a typical endothelioma starting in the dura of the cord. I was unable to obtain the clinical history because it was a coroner's case.

(Dr. Clark E. Brown, Philadelphia.) Recently we have had a postmortem on a case very similar to the one described by Dr. Karsner in which there was what we thought to be the original tumor in the apex of the right lung, or in the subclavicular fossa on the right side; it was impossible to say which. It was very destructive in nature and consisted of a soupy, grayish material. What we took to be metastatic involvement of both adrenals was found, and also involvement of the mesenteric nodes and the submucous tissue of the stomach. Histologically the predominant cells were multinucleated, pear-shaped and uninuclear spindle-shaped cells, which we took to be those of spongioblastoma multiforme. We had some discussion with Dr. McFarland about this tumor and he was inclined to agree with that diagnosis at first, but on further thought he showed us some sections of epidermoid carcinoma which looked very much like the cells of this tumor.

(Dr. William Carpenter MacCarty, Rochester, Minn.) Was the posterior nares examined in this case? I have seen several intrathoracic tumors where the primary tumor was in the posterior nasopharynx.

(Dr. Victor C. Jacobsen, Albany.) I did not realize that this tumor was so rare. One case, which I have included in an increasingly large series of primary carcinomas of the lung, is similar to the case reported and very much like Dr. Karsner's. X-ray showed invasion of the ribs and vertebrae by the tumor. I made the first diagnosis of epidermoid carcinoma from biopsy material removed from just alongside the vertebra. At autopsy the tumor was present in the right upper lobe, toward the midline. I took the lung out carefully and injected it with Kaiserling solution. Later, when it was well fixed, I dissected out the bronchi leading to the region of the tumor and found the right eparterial bronchus disappearing in the tumor. Sections at that point showed a transition from bronchial epithelium to the squamous epithelium of this type of cancer. I have included it as a case of epidermoid carcinoma of the bronchus, in which category about 32 per cent of all lung carcinomas belong.

(Dr. Kornel L. Terplan, Buffalo.) With regard to Dr. Karsner's discussion, I should like to mention that malignant neuroblastic tumors of the peripheric nerve, especially of the sympathetic nerve, are very unusual in adults. A possible neurogenic origin, however, may be suggested by rosette-like arrangements of the immature blastoma cells, if we fail to demonstrate by all specific methods any more mature nervous element. Was there such an arrangement in your case, Dr. Karsner? Otherwise, I believe that Dr. Clarke's tumor may have originated from the pleura or from the lung itself. Sometimes carcinomas of the lung may grow into the pleural cavity, similar to a mushroom, and spread along the pleural surface. On the other hand, primary tumors of the pleura may differentiate into

epithelial tumors, as well as more immature mesenchymal or mesothelial tumors.

(Dr. Paul R. Cannon, Chicago.) I wish to mention the occurrence of one of these types of tumors in an autopsy performed by Dr. Steiner of the University of Chicago. The patient had a tumor in the left pulmonary sulcus with a Horner's syndrome and roentgenological findings consistent with a diagnosis of pulmonary sulcus tumor. Postmortem examination, however, revealed a typical adenocarcinoma, rich in mucin, located in the left pulmonary apex and extending into the pleura. Metastases were also present in the pelvic bones. Dissection of the primary growth suggested that the tumor had originated in an area of fibroplastic tuberculosis in the apex and we concluded that the tumor was a bronchogenic carcinoma.

(Dr. Clarke, closing.) This discussion seems to support my closing suggestion that the lesion is not a pathological entity. So far as the histological diagnosis in my case is concerned, sections were studied by Dr. Ewing, Dr. Mallory and Dr. Wolbach. They all arrived at the same diagnosis, epidermoid carcinoma, although they did not agree as to where it might originate. On my way here I stopped at Montreal and the sections were seen by Dr. Vernon Cone, who was of the opinion it did not originate in any nervous structure.

In answer to Dr. MacCarty's question, the nasopharynx was examined.

I was unable in this case to trace any connection with the bronchus. The lung involvement was so superficial that only a very small bronchiole could possibly have been involved.

PINEAL TERATOMA WITH UNUSUAL ADAMANTINOUS FEATURES. Samuel Judd Bochner and John E. Scarff (by invitation), New York City.

Abstract. Pineal teratomas are rare, only 14 completed cases having been reported, of which only 2 are in the American literature. Another case is here added, presenting unique dentigenous features hitherto not reported in the literature.

J. S., a boy of 9 years, was admitted to the Pediatric Service of the New York Post-Graduate Hospital with the history that for the past 2 years he had suffered occasional attacks of sharply localized frontal headaches, associated with extreme drowsiness and followed by apparent complete recovery. On the morning prior to admission a more severe attack occurred, accompanied by convulsive tremors and unconsciousness. On the ward he suffered another such seizure, associated with irregularity of the pulse, Cheyne-Stokes' respiration and a semicomatose state terminating in death.

The unusual X-rays first attracted attention to this case. These seemed to show well advanced tooth structures in the region of the third ventricle.

Autopsy revealed a semicystic tumor filling and distending the third ventricle and markedly compressing the adjoining structures. It measured 5 by 5 by 3.5 cm. Section revealed a number of essentially adult teeth. Microscopic studies of these areas presented all stages of dental evolution from the simple nests of ameloblasts and characteristic adamantinous structures of the primitive enamel organ to the fully formed tricuspid structures, including enamel, dentine and pulp. The adamantinomas, described by Frazier and Alpers and others, differ fundamentally from this tumor in that no adult forms are reported in their cases beyond irregular and somewhat questionable enamel masses. The tumors in their series reputedly arise from the craniopharyngeal pouch, contain only

buccal elements and may be classified as epidermoids. This case, on the other hand, presents a multiplicity of structures derived from all three germ layers, both embryonal and adult in form. There are connective tissue elements varying from dense collagenous structure to the open framework of areolar tissue. Masses of hyaline cartilage are frequent, in some instances presenting early ossification, in others completely formed lamellar bone with Haversian systems. In other areas primitive membranous bone is noted. The many cysts and interconnecting spaces are lined by tall, columnar, ciliated epithelium, rich in goblet cells and forming numerous out-pouchings. These vary from simple to compound racemose seromucinous gland structures, constituting a picture remarkably like that of the nasal mucosa. In one area thyroid-like tissue is present. Widespread throughout the mass and forming a large portion thereof are well defined nerve tissue elements, both glial and ganglionic. Pineal tissue also is scattered through many areas, presenting the picture of embryonal undifferentiated structures, as well as the characteristic groupings seen in infancy. The fibrosed substance of the so-called involuted pineal body is not found.

This observation of pineal and nerve tissues in close association is of interest in view of the wide difference of opinion existing among different workers as to the presence or absence of nerve and glial elements in the pineal body.

Careful questioning of the family failed to reveal any psychical, sexual or developmental abnormalities. Past studies would indicate that adenomas of the pineal tend to retard sexual development and maturity (Marburg), and that teratomas induce sexual precocity and maturity (Askanazy). It is possible that in this case a balanced result of two antagonistic influences is manifest.

Discussion

(Dr. Harry C. Schmeisser, Memphis.) It may be of interest, in relation to this case, to bring to the attention of the members of the Association that I am exhibiting downstairs, among my photographs of clinical and anatomical lesions, a photograph of a brain showing a dermoid cyst in the fourth ventricle which was the cause of an internal hydrocephalus.

LIPOID HISTIOCYTOSIS WITH TUMOR-LIKE INVOLVEMENT OF SUBCUTANEOUS FATTY TISSUE, AND WITH DIABETES INSIPIDUS. K. L. Terplan and (by invitation) Roger S. Hubbard, Buffalo, N. Y.

Abstract. An exceptional case of lipoid histiocytosis was observed in a white woman, 34 years of age. This case differed from those reported as Christian-Schüller's syndrome in the presence of tumor-like infiltration of large areas of the subcutis, and in the absence of bone tissue changes and of exophthalmos. Marked polydypsia and polyuria had been present. The appearance of the chest clinically suggested a diagnosis of malignant invading blastoma or Hodgkin's disease. Deposits in the dura, with sinus thrombosis, small granulomatous structures in the serosa and somewhat similar structures in the lungs, pointed to a relation to Christian-Schüller's syndrome. Involvement of the bone was restricted chiefly to the bone marrow and was especially marked in long bones, less marked in ribs and vertebrae, producing marked anemia with signs of erythropoiesis, as in carcinomatous invasion of the marrow. Other important findings included thrombosis of the splenic vein with extensive older infarction, thrombosis of large iliac and pelvic veins extending into the inferior vena cava,

marked atrophy of thyroid (6.3 gm.) and moderate storage of lipoid in the mediastinum, retroperitoneal fat tissue and peripancreatic tissue. The mesenteric fat was not altered.

Histological analysis pointed to gradual replacement of the subcutaneous fat cells by huge foamy cells, stored with double-refracting lipoids, and subsequent reactive inflammatory changes with fibrosis. From stains with sudan III, Nile blue sulphate, and Weigert hematoxylin (L. Smith), most of these lipoids were cholesterol esters, particularly, according to Böhminghaus, cholesterol oleates. Histological study pointed in some areas to the appearance of huge cholesterol masses in the otherwise structurally normal fat cells. This deposition was especially plain in the fatty islets which were still preserved on the borders of large masses of foamy cells and granulation tissue. The endothelial cells in the liver, spleen and lymph nodes did not show storage of lipoid. The spleen showed changes in connection with venous thrombosis and its consequent hemorrhages. The pituitary showed practically complete fibrosis of the posterior lobe with some inflammatory cells, especially plasma cells. When the diagnosis of unusual storage of double-refracting lipoid in the subcutaneous tissue was made from biopsy it was already suggested that the case differed from those ordinarily attributed to a disturbance in cholesterol metabolism. Even though the lipoid infiltration of the subcutaneous tissue had markedly increased during the patient's stay in the hospital, at no time was the blood cholesterol increased. Atheromatosis was very slight. Lipoid content of adrenals was normal.

The aforementioned clinicopathological findings obviously assume a unique character when this case is compared with the usually described example of Christian-Schüller's syndrome. It must be mentioned that huge tumor-like nodules composed of cholesterol fatty acid esters were described by Proescher and Meredith in the skin and abdominal cavity of a 32 year old woman under the heading "multiple myxocholestolipomata." However, this last case did not show cholesterol deposition in other organs, or diabetes insipidus.

In the dried tumor tissue 57.5 per cent was acetone-soluble lipoid. In this lipoid 57.4 per cent was cholesterol ester, apparently almost wholly cholesterol oleate, which was purified by crystallization from methyl alcohol and identified by analysis and a melting point determination. Other lipoid constituents found in the material analyzed included free fatty acid (15.7 per cent), neutral fat (13.8 per cent), free cholesterol (3.9 per cent), and phospholipoid (2.6 per cent).

PSEUDOCANCER OF THE STOMACH. Anatole Kolodny, Sioux City, Iowa.

Abstract. An inflammatory mass was found in the stomachs of two patients. In both cases the clinical symptoms and the X-ray findings suggested a carcinoma of the stomach. At operation a wide resection of the stomach was done in each case. Grossly the lesion suggested a malignant growth, but the histology proved the lesion to be inflammatory. The patient who survived the operation is now well, nearly 3 years after the operation.

Discussion

(Dr. James Ewing, New York.) I am very much interested in this communication of Dr. Kolodny. I do not think, however, that he has given us sufficient histological evidence to determine whether he may still be dealing with one of those types of cancer of the stomach which have a tendency to show very

marked regression and atrophy of the cancer cells or not. I should like to have an opportunity to study his sections in detail.

(Dr. Kolodny.) I shall be very glad indeed to give the entire pathological material to anybody desiring to study it. I shall be very glad to have Dr. Ewing's opinion. I did not want to enlarge on the gross description of the specimen removed because of lack of time. However, the removed lymph nodes, which at first suggested malignant involvement, proved on final section to be purely inflammatory in nature; the normal portion of the stomach was greatly swollen, so that on the operating table it was difficult to determine where the tumor stopped. The matting together of the lesion with the pancreas, the duodenum and omentum was so widespread, much more so than one sees in carcinoma, that these findings, together with the histological appearance of the tumor, led us to believe that we were dealing with a benign lesion.

SPLEEN WITH ARTERIES ENCLOSED BY VEINS. Harry C. Schmeisser, Memphis, Tenn.

Abstract. The patient, C. L., 50 years old, a male negro, was admitted to the Memphis General Hospital, May 20, 1933, and died the following day.

The clinical and the autopsy findings had no bearing on the subject in question, except that an extensive examination of other arteries and veins grossly and microscopically showed no anomaly. There was also a moderate degree of generalized arteriosclerosis and a bilateral bronchopneumonia. To the latter his death was attributed.

The spleen was of average weight and size and of the usual external appearance. The splenic artery and vein outside of the spleen were normal. As the branches of each entered the hilum and ran in the trabeculae the artery became enclosed by its companion vein. This was clearly observed by serial section and dissection along the course of a particular pair of vessels, exposing them in long and cross-section. As the vessels approached the periphery of the spleen they separated on leaving the trabeculae.

Sections for microscopic study were taken of paired vessels and showed an artery and a large vein within a trabecula. The various layers of the wall of the artery were recognized and over a portion of its adventitial surface it was attached to an outer circular tissue which was either an arch of the vein and trabecula, or trabecula only. Here and there were endothelial-lined blood cells containing spaces, which suggested the remains of the vein lumen. Surrounding the remaining larger part of the adventitial circumference of the artery was the endothelial-lined lumen of the vein, which was continuous by probing and sight with the lumen of the splenic vein. In a similar manner it was proved that the microscopic arterial lumen was continuous with that of the splenic artery.

We were either dealing with a pseudo- or true enclosure of artery by vein. By pseudo-enclosure is meant that the artery running parallel to the vein in the trabecula had pressed into the vein to such a degree as to be almost entirely surrounded by the same and give the appearance in the gross that the artery was actually lying within the lumen of the vein. By true enclosure is meant that the artery had penetrated into the lumen of the vein. In this case it will be necessary to assume that the endothelium which lined the lumen of the vein had proliferated over most of the circumference of the adventitia of the artery, and had disappeared in places where the same was attached to the intima of the vein. A more detailed study will be made.

THE TYPES OF ARTERIOSCLEROSIS. Oskar Klotz, Toronto, Canada.

Abstract. The modern conception of the subject of human arteriosclerosis was reviewed with the aid of lantern slides. The author in the presentation of his subject made a plea for more careful observations at the autopsy table of arterial lesions, their nature and distribution. There is ample opportunity for all pathologists in participating in studies upon arteries, and there is still a variety of lesions in intima, media and adventitia which remain to be described and correlated with systemic disturbances. It is necessary, however, that all speak in the same "scientific tongue" and apply a given terminology with a definite and specific meaning. It was pointed out that Lobstein (1833), who coined the term arteriosclerosis, gave it a clear definition, from which we are not privileged to divert according to our own notions. So, too, the term atheroma has a definite meaning as indicated by Haller (1756). Atheroma is not synonymous with arteriosclerosis, nor does atheroma refer to any lesion containing fats and lipoids. The lesion lies in the intima, and consists of a cavity containing free fats, lipoids and cholesterol compounds in quantity sufficient to recognize a grumous mass. Fat in cells is not atheroma. Of late years the term atherosclerosis (Marchand 1904) has been badly misused. It is only necessary to observe the use of this word in a recent book on arteriosclerosis, and note how each of many authors adds confusion to our literature by his careless application of the term to realize that the word has lost its meaning. Two or three decades ago Virchow's term "chronic endarteritis" (1856) suffered similar mishandling. It is evident that much misunderstanding by different investigators has arisen through improper use of definite terms. Investigators must be equally careful in their observations and in their manner of recording those observations.

The demonstration of the types of arteriosclerosis gave in review the important lesions found in all three coats of the arteries. The importance of the scleroses in the arteries of the heart, kidney and brain was stressed, but it was also shown that the vascular lesions in these three organs are not identical, nor do they arise under similar conditions. The renal arteriosclerosis particularly has characteristics of its own, showing an inflammatory background and not developing the quality of an atherosclerosis. The progressive atrophy of the media with fibrosis, as seen in the cerebral vessels opposite to the nodular internal scleroses, is distinctive for this group of vessels. Much careful analytical work must now be carried out to distinguish properly the qualitative differences in the several types of intimal sclerosis.

The discussion of arteriosclerosis must include lesions of the media. Pathologists are only awakening to the importance of the pathological processes which are found in this layer. Inflammatory processes, as found in rheumatic fever, sepsis, typhoid and other infections lead to scars which are later recognized as scleroses, in association with intimal thickenings overlying them. In other cases the middle coat of the artery suffers degenerations of a mucoid, fatty or calcareous kind. The mode of origin of these separate processes is not known and offers opportunity for extended research.

Much investigation should be encouraged for a better knowledge of the lymphatic drainage of the arterial wall and the innervation of the arteries. A clearer understanding of the normal structure and function of the component tissues of the arteries would assist materially in interpreting the pathological events which take place in these vessels.

The author also reviewed the varieties of scleroses that had been induced in experimental animals. These lesions have been of great interest in indicating the manner in which they arise in animals, but care must be taken in applying these findings to the human. The hypercholesterolemia induced in rabbits leading to lipoid deposits in the arteries is a mechanism quite different from that related to similar lesions in man. A wide scope of studies awaits further investigation and calls for the combined interest of the biochemist and pathologist.

COMPARATIVE CHEMICAL AND HISTOLOGICAL EXAMINATIONS OF AORTAS FOR CALCIUM CONTENT. Samuel R. Haythorn and (by invitation) Fred A. Taylor, Helen Whitehill Crago and Anna Zoe Burrier, Pittsburgh, Pa.

Abstract. Parallel chemical and histological examinations were made on fifty-two aortas from patients of various ages, and the findings compared. The routine consisted in taking three samples from each aorta and in submitting them both to chemical analysis and to microscopic study by paraffin and frozen section methods. The samples were taken respectively from the ascending, thoracic and abdominal portions of each aorta. The chemical samples were taken from areas bordering on each side of the pieces for microscopic examination. When possible, exactly 10 gm. of aorta per sample were used. The aortas were stripped of adherent tissues, were submitted to the Corey-Denis method for calcium content, and reported in milligrams of metallic calcium per 100 gm. of wet aorta.

The results were grouped according to the ages of the patients in decade periods. In the groups under 40 years of age the chemical and histological findings were consistent, save for two aortas; one of these was from an infant with severe rickets and the other from a 38 year old patient with luetic aortitis. Thirty-two aortas came from patients over 40 years old. With each succeeding decade the comparative findings were more inconsistent and less easily interpreted. The average calcium content of 11 aortas in the 41 to 50 age group was 234.5 mg., of 10 aortas in the 51 to 60 group was 590 mg., and of 10 aortas in the 61 to 70 group was 553 mg. Two atheromatous aortas, both from 60 year old patients, explained the fact that the 51 to 60 group was higher than the 61 to 70 group.

An outstanding feature of the work was the great variation in the amounts of calcium found at different levels of the same aorta where there was a different degree of atheroma present in the given levels. Throughout all three of the older age groups the heaviest deposits were found in the segment above the bifurcation. The highest amount found was in an abdominal segment presenting numerous large calcium plates with ulcers about them and was 2312 mg. In a general way the calcium plates yielded the greatest amount of calcium, the combined cystic nodules with microscopically demonstrable medial calcium next, and those with medial calcification third, although there were none of these in our series that were not complicated by nodules. The amounts found in intimal cysts were so inconsistent that no conclusions were drawn.

None of the histological methods gave more than a general idea of what the chemical report was likely to be. Silver nitrate, while not considered specific, was perhaps the best indicator of total calcium, particularly where the test was made in bright sunlight. A modification of Cameron's alizarin stain yielded beautiful preparations, but failed to stain all of the calcium present.

THE RELATION OF CHOLESTEROL TO ATHEROSCLEROSIS. Timothy Leary, Boston, Mass.

Abstract.

(*A*) *Human Coronary Sclerosis:* Study of the lesions of coronary sclerosis and thrombosis demonstrates: (1) that atherosclerosis is a disease and not the inevitable consequence of age, since it occurs in early life and is frequently highly selective in its localization. (2) That the process is metabolic and not inflammatory. Such lymphoid cell infiltration as may be found in coronary lesions is on the whole infrequent, is accidental and not necessary, and is a late phenomenon when found. (3) That in the young human being progressive fibrosis is the usual picture in narrowing of the coronary lumen. Lipoid cells filled with cholesterol esters are deposited in the intima beneath the endothelium, but are replaced by young fibrous tissue before any considerable accumulation of these cells can occur. The deposit of the lipoid cells is progressive or cyclic, and a growth of young connective tissue replaces them *pari passu*, until the lumen is markedly narrowed. (4) That in the aged, large collections of lipoid cells arise with a minimal amount of fibrous tissue support. The delicate fibrous tissue (reticular) partitions between these cells are dependent upon a lymph or plasma diffusion, by imbibition through the endothelial layer from the blood in the arterial lumen. As the cell masses accumulate the nutrition of the cells tends to suffer and massive necrosis occurs, followed by liquefaction, producing so-called atheromatous "abscesses." (5) That the standard cause of death in young individuals is coronary thrombosis. This is due to necrosis arising in the subendothelial layer of the new fibrous tissue of the intima and extension of the necrosis to the endothelium. Apart from thrombosis, death in this group may be due to narrowing of the lumen and coronary insufficiency, under conditions of stress. The circulation through the narrowed vessels, while adequate under ordinary conditions, becomes inadequate under increased demands for blood. (6) That the standard cause of death in older individuals is the rupture of an atheromatous "abscess" into the coronary lumen, with secondary thrombosis. Rupture from the lumen into the atheromatous pockets also occurs. Death in this group may also be caused by coronary insufficiency (due to the narrowed lumen), under stress, without rupture of an atheromatous "abscess" or thrombosis. (7) Lesions of the elastica, media and adventitia are secondary, apart from possible stress effects. The primary process is lipoid, metabolic and intimal.

(*B*) *Human Coronary and Experimental Rabbit Atherosclerosis:* Comparative study of the lesions of human coronary and rabbit experimental atherosclerosis demonstrates: (1) that the lesions of human coronary sclerosis may be reproduced in rabbits by feeding cholesterol. Intimal sclerosis (atherosclerosis) occurs spontaneously in much less than 1 per cent of young rabbits. It was produced in 92.6 per cent (25 out of 27) of young rabbits in our series fed with cholesterol. (2) That in young rabbits, as is true in young human beings, intimal fibrosis in arteries is the standard lesion, atheromatous "abscess" formation the highly exceptional process. The fibrosis, *i.e.*, the reparative response, which arises in atherosclerosis in the arteries of the young is, therefore, a reaction of youth and not of species.

Any metabolic agent capable of causing atherosclerosis must have been a part of the diet of human beings from the earliest times, since atherosclerosis has been found in the bodies of mummies. We think of cholesterol in terms of blood cholesterol or bile cholesterol. This, however, is only the mobilized

cholesterol. Cholesterol is a substance required by every animal cell. Starling conceives of it as the stable substance of the animal cell — the framework — in the interstices of which the more labile substances undergo their metabolism. The greatest need for cholesterol arises in times of most rapid cell production. Egg yolk is intended for the embryo. Milk is intended for the infant. The increased blood cholesterol in pregnant women is mobilized to supply the fetus with this substance essential for its cells. Man is the only animal that ingests eggs and milk throughout its lifetime. Man is also the only animal, as far as is known, that dies in early life of coronary thrombosis and exhibits atherosclerosis almost universally with age.

The analogy of this metabolic disease to diabetes is close, even though cholesterol, in contrast to sugar, is a substance of difficult metabolism, and tends to accumulate within the body, *i.e.*, to be stored. As in diabetes, inheritance of a poor cholesterol metabolism results in manifestations of the disease in early life. As in diabetes, the advent of age, with the development of inefficiency of the cholesterol metabolism, is associated with more frequent late evidences of the disease.

Recent human experience in diabetes supports the experimental rabbit findings following cholesterol feeding. During and following the period when excessively fat diets were used in the treatment of diabetes, there was so great an increase of atherosclerosis that Shields Warren wondered whether the increase was due to diabetes or to the treatment of the disease. X-ray examination of the legs of children disclosed evidence of calcified atherosclerotic leg arteries, and xanthomas were common. Recent reports from the Joslin clinic, under a lower fat diet in the treatment of diabetics, indicate that xanthomas no longer appear, and X-ray evidence of calcified leg arteries in children is lacking.

HISTOPATHOLOGY OF THE CONDUCTING MECHANISM IN A HEART WITH VARIOUS DEGREES OF BLOCK DUE TO ARTERIOSCLEROSIS. Mortimer Cohen (by invitation), Pittsburgh, Pa.

Abstract. Degeneration of the conducting mechanism is associated with sclerosis of the arterioles supplying the system. The sclerosis involves the intima primarily with definite encroachment upon the lumen. This interference with the blood supply results in shrinkage of the component parts of the system. The extent of the shrinkage, however, is not the same over the entire mechanism. In the sino-auricular node the involvement is moderate; it is greater in the auriculoventricular node and in the main bundle. The left branch is reduced to a few fibers. The shrinkage is due to actual disappearance of some individual fibers and distinct narrowing of the surviving fibers.

The connective tissue is increased in the mechanism itself, but the greatest increase is seen in the fibrous portion of the interventricular system.

The electrocardiogram demonstrates various degrees of block varying from latent to complete. This variation may depend on the ability or failure of the surviving damaged fibers to transmit impulses.

Sclerosis of the arterioles is present elsewhere in the heart with degeneration and replacement fibrosis. This damage is pronounced in the interventricular septum. Here the thebesian veins are distended, apparently attempting to supply the injured area with blood.

Discussion of Papers by Drs. Klotz, Haythorn and co-authors, Leary, and Cohen

(Dr. Harry C. Schmeisser, Memphis.) In relation to Dr. Cohen's paper, permit me to call attention to a very interesting case of heart block caused by gumma of the septum, published by Bridgman and myself in the Johns Hopkins Hospital Reports, 1916, in which the complete clinical and pathological findings could be compared. The lesion, in this case, was readily seen with the naked eye, measuring 4.5 by 3 by 1.5 cm., and histologically was found to involve the bundle of His. Heart block due to arteriosclerosis, as presented by Dr. Cohen, required a more detailed histopathological study.

(Dr. Howard T. Karsner, Cleveland.) Many points of interest have been drawn to our attention by the excellent studies presented this afternoon. In order to initiate further discussion, there are a few items to which I wish to refer. As Secretary I take this opportunity to express publicly to Dr. Klotz the warm thanks of the Council for the clarity and inclusiveness of his paper, the summary of many years of intensive study of arteriosclerosis. Of great interest is Dr. Leary's material, carefully studied and beautifully presented. In regard to his work I wish to express the view that the influence of syphilis on coronary arteries cannot be dismissed, especially when as the result of Moritz's studies of 8 cases of syphilitic aortitis a lesion of coronary arteries, which occurred within the first 2 or 2.5 cm. from the origin, was identified as syphilitic in all the cases examined (*Arch. Path.*, 1931, **11**, 44). It is undoubtedly true, as Dr. Leary points out, that there must be significance to the lipoid deposits in the development of coronary and other forms of arteriosclerosis, but whether this represents cause or effect is at the present time a matter of interpretation. I have studied carefully the coronaries in more than 50 rheumatic hearts and am impressed by the constancy of significant lesions of these vessels (*Am. Heart J.*, in press). I do not mean to imply that these lesions are specifically rheumatic, but regard them as manifestations of infectious disease. Reference has been made to the Macy Foundation Survey of Arteriosclerosis, edited by Cowdry. In this volume, McCallum expresses the view that there is little ground for regarding infectious diseases as of importance in the production of arteriosclerosis. As a result of my studies of rheumatic hearts, the studies of Klotz and of many others, I think that consideration must still be given to the significance of infectious diseases in the development of arteriosclerosis in general and coronary sclerosis in particular.

Dr. Cohen's studies clearly indicate the importance of arterial disease as a cause of heart block. Although in the earlier case reports gumma was emphasized, it now is apparent that coronary sclerosis is a considerably more frequent cause of heart block than is gumma. Dr. Cohen's demonstration of the communication between arterial channels and the thebesian system is striking. While I do not wish to state that thebesian circulation is not of importance in maintaining myocardial nutrition, nevertheless, the rediscovery by Moritz and his associates of Langer's observations showing anastomosis between coronary arteries and vessels in pericardium and mediastinum must be considered (*J. Exper. Med.*, 1931, **56**, 919, 927). It is at least possible that these extracardiac anastomoses of the coronaries may play a part in nutrition of the heart muscle when intracardiac coronaries are reduced in lumen or occluded.

(Major V. H. Cornell, Washington.) There is one thing I should like to ask Dr. Cohen, and that is if the hearts were studied in serial sections. I ask this

because I have recently been working with Dr. Yater on several such cases (we have a series at Washington). In his summary of the literature he states that many cases must be discarded because complete material has not been studied. Perhaps personally I did not appreciate that as fully until a few days before I left Washington when we saw a case without apparent severe coronary involvement, but with calcification of the mitral valve at the base of the aorta with a spur jutting out just beneath the mitral leaflet. On serial section after running through several trays the bundle showed a small spot of calcium at the center. This rapidly grew to the full size of the bundle with complete obliteration of the bundle and then disappeared in 170 serial sections, which approximated 1.5 mm. of the bundle. You can see how readily such a lesion would be missed if serial section was not used.

Another point which occurred to me was that although in the case presented we see so much sclerosis of the coronary branch, we do see a compensatory circulation, and the question arises as to whether the lesion of the bundle is due to circulatory deficiency, or whether it is not a part of the general fibrosis occurring in all the tissue around it, and whether that fibrosis itself is primarily due to circulatory disturbance or to some other factor.

(Dr. E. T. Bell, Minneapolis.) There is one form of arterial disease which has not received as much attention as it deserves, I think, and that is fibrosis of the media. If you study the media of the muscular arteries you find in young persons there are just a few collagenous fibers between the muscle cells. In middle age, in the forties, there is a decided increase in the collagenous fibers, and in advanced age, seventy to eighty, the muscular arteries of the extremities, particularly, are almost completely replaced by collagen, so that commonly there are no muscular fibers left. That is a very striking alteration and can be brought out beautifully with the azocarmine stain, and is not related to any factor, so far as we know at present, except age. One is impressed in studying arterial disease with the variations of the anatomical type in different arteries. The changes in the muscular arteries are quite different from those in the elastic arteries. In the muscular arteries the changes are chiefly a medial fibrosis, a medial calcification, and in the elastic arteries the outstanding changes are intimal. The most prominent feature of arterial disease, what we call arteriosclerosis, is age, and that seems to be much more important than anything else. That there are other things is brought out by the fact that we frequently have arterial disease, even fatal types, in young persons, which encourages us in the search for the factors that will influence it. We must not forget that one thing which influences intimal disease is diabetes. If you have had much experience in postmortem examinations you know that intimal disease is usually much more prominent in diabetics than in non-diabetics of the same age, and disordered metabolism is therefore one possible etiological factor.

(Dr. N. C. Foot, New York.) It might be well to point out in connection with animal experimentation along these lines the importance of knowing the genetic history of the rabbits with which you are working. Out in Cincinnati, Zeek in our Department attempted to reproduce the Newberg experiments on a number of Belgian rabbits without any result, except that the renal lesions were reproduced. The aortas remained unaffected. Later on the rabbits in our hutch were found to develop arteriosclerosis spontaneously, and Zeek found that one buck in particular seemed to possess the faculty of producing progeny, all of which developed spontaneous arteriosclerosis; so that, had we used that particular strain to work with, we would undoubtedly have produced very nice arterio-

sclerotic lesions, which, had we not known the history of the animals, might have been explained to our satisfaction on totally different grounds and had nothing to do with the experimental work in hand. Therefore, it is very important to know all about your rabbits' family histories before you begin on cholesterol feeding experiments, Newberg experiments, and the like.

(Dr. Leary.) First, with reference to syphilis of the coronary, I have examined a large number of cases of syphilitic aortitis and I have yet to see an example of syphilitic disease of the proximal portions of the coronaries, that is, of the portion of the vessels which lie beneath the epicardium. Even Warthin, who found syphilitic focal lesions universally, admitted that syphilitic lesions of the main coronary arteries were rare.

With reference to rheumatic lesions of the coronaries, it is possible that they may have a primary influence in some cases. The standard lesion of coronary sclerosis, *i.e.*, atherosclerosis, can and does appear usually independently of infection as such. Infection may lead to coronary stresses, which in turn may favor the deposit of cholesterol, but the absence of evidence of inflammatory reaction in early atherosclerotic processes negatives the claim that infection is a necessary or a common factor.

Dr. Bell said that the outstanding lesions in the muscular arteries were fibrosis of the media. That is not my experience with relation to the coronaries. Secondly, he brought up the relation of atherosclerosis to diabetes. I think the factor of diet in the experimentation which was carried out in the treatment of diabetes supports completely the rabbit experimentation we have been carrying on. You will remember that excessively fat diets were used some years ago, which led to such an increase in arteriosclerosis that Dr. Warren wondered whether the increase was due to diabetes, or to the treatment of the disease. Since the high fat diets have been discontinued, atherosclerosis is not so outstanding, the xanthomas which were common disappeared, and it is impossible now in the same clinic to get X-ray evidence of children's arteries which have calcified.

With reference to Dr. Foot's remarks, proper controls take care of that situation. Our controls were all negative, save that one rabbit showed two minute foci of medial sclerosis in the aorta.

(Dr. Bell.) The muscular arteries I had reference to are those in the extremities and abdominal viscera. It does not apply to the coronaries.

(Dr. Klotz.) I wish to thank you for the very kind reception you have given to the presentation of my paper. I also wish to extend my congratulations to Dr. Leary for the demonstration of a beautiful series of slides of coronary arteriosclerosis. Dr. Leary has clearly brought to our attention the frequency with which severe coronary scleroses are encountered in the relatively young as well as old individuals. The lesions which are encountered in the fatal cases are very similar in character, showing stenosis of the lumen of the vessel and marked lipoid degeneration of the intimal lesion. It is, however, difficult to determine the antecedent history of these human lesions at such a late stage in their development. Dr. Leary accentuates the importance of the finding of fatty materials in these scleroses. I am sure, however, that Dr. Leary does not wish to convey the idea that all coronary lesions arise in this manner, and that he will admit the development of others on an inflammatory basis. Such a one I illustrated in a child of 9 years, who after recurrent attacks of tonsillitis developed a glomerulonephritis along with a well marked coronary sclerosis. Another case showed similar endarteritis without lipoid changes in a girl of 14, who

had suffered a succession of severe infectious diseases during 2 years preceding death. It is very clear that our studies in the future must lay more stress upon the arterial lesions at their commencement rather than attempt to indicate the course of the lesions after they have attained their late characteristics after middle life.

I am sorry that Dr. Bell has suggested to us that age is a cause of arteriosclerosis. It is as though age were a crime. I am sure he did not mean that. It is true that with advancing years we accumulate the scars of previous damage and thus for the demonstration of numerous and well marked examples of arteriosclerosis we seek the tissues of elderly individuals coming to autopsy. Age, however, has not been a factor in producing these lesions. The wear and tear of life upon tissues is not so important as other essential factors which have a specific influence upon certain structures. It is, however, pleasant to think that there is no truth in the old belief that alcohol produces arteriosclerosis or that smoking brings about lesions in the human aorta. The experimental evidence that nicotine acts upon the aorta of some of the lower animals cannot be applied to the human subject. The manner of these experiments is entirely different from anything that happens to man.

Dr. Foot has brought up a very important point respecting the use of experimental animals in the study of arteriosclerosis. Nearly every one of the lower animals that is used for experimental purposes develops its own type of arteriosclerosis, and one must be very careful in distinguishing these lesions from such that may be induced by the experiments. On the other hand, too, as has been stated by Dr. Foot, some of these animals develop arteriosclerosis with great ease and at periods in their life when not expected. Some strains of animals show scleroses in greater frequency than others belonging to the same species. Furthermore, in the study of arteriosclerosis the rabbit has been utilized most commonly for experimental purposes. This herbivorous animal is so different from the human that the greatest care must be taken in interpreting the experimental results. I am far from satisfied that the animal experiments, indicating cholesterol compounds as important factors in the production of arteriosclerosis, can be accepted for the interpretation of the human lesions. The cholesterol metabolism in the rabbit and in the human are two entirely different processes. It is my belief that the cholesterol-lipoid substances are only an index of the damage imposed upon the arteries, and that they do not represent the factors causing the lesion. In the future it may be well to turn from the rabbit and carry on experiments in the monkey or in other omnivorous mammals. In each case it will be necessary to familiarize oneself with the natural diseases arising in these animals as well as with the normal metabolism concerning various substances. The experimental work which has heretofore been carried out on a few of the lower animals has been interesting and has assisted in understanding reactions in the arterial wall. The experiments, however, are still inconclusive in respect to an appreciation of the human disease.

CARDIOVASCULAR RENAL CHANGES ASSOCIATED WITH BASOPHILIC ADENOMA OF THE ANTERIOR LOBE OF THE PITUITARY. H. E. MacMahon, Boston, Mass.

Abstract. In unravelling the rather complex clinical syndrome of pituitary basophilism one finds recurring with remarkable constancy a group of signs and symptoms indicative of cardiovascular renal disease; a greatly elevated blood

pressure, an enlarged heart, associated with frequent headaches and disturbances in vision. These may be present over a period of months or years, to be complicated at a later period by signs of renal decompensation, namely, inability to dilute and concentrate fluids, and a progressive retention of nonprotein nitrogen substances in the blood stream. The urine will reveal albumin, casts of various types and red blood cells.

A study of such cases both clinically and from the standpoint of pathological histology has shown that this cardiovascular renal disease simulates that described by Volhardt and Fahr as malignant nephrosclerosis.

Discussion

(Dr. Howard T. Karsner, Cleveland.) Will Dr. MacMahon state whether or not the acute necrotizing arteriolitis is present in all the cases of basophilic adenoma of the anterior lobe of the pituitary that he studied?

(Dr. MacMahon.) Acute necrotizing arteriolitis has not been a constant feature. I have seen it in 3 cases of basophilic adenoma of the anterior lobe of the pituitary. These patients showed signs and symptoms of advanced pituitary basophilism (Cushing's syndrome), including marked hypertension, renal decompensation of varying degree with red blood cells and albumin in the urine. Two days ago I received some kidney sections from Dr. Hussey, from a patient of Dr. Cushing showing signs of pituitary basophilism. Clinically there were no signs of renal decompensation and the urine contained no blood. Histologically the only remarkable lesion in the kidney was a vascular hypertrophy and marked swelling of the basement membrane of the small arterioles.

(Dr. Kornel L. Terplan, Buffalo.) The relation of the basophilic cells in the hypophysis to hypertensive disease is of great interest, although we have not arrived as yet at a clear conception of the etiological rôle of the basophilic cells or basophilic adenomas in this connection. From the papers of Kraus, Berblinger and Zeynek we know that the number of basophilic cells is considerably increased in different diseases, especially in those with a high cholesterol level, such as marked essential hypertension and chronic glomerulonephritis. This also has been observed in cases of severe obesity, regardless of whether or not they showed hypertension. In 1 case of basophilic adenoma of the hypophysis, which I saw in Prague, the only one I recall, there was no associated hypertension.

(Dr. MacMahon.) I think it is quite possible that one may find a basophilic adenoma of the pituitary in the absence of vascular renal changes. It is quite true that one finds other examples of adenoma appearing in glands of internal secretion without showing any clinical manifestations. In the case of the basophilic adenoma of the anterior lobe of the pituitary showing signs of pituitary basophilism, the cardiovascular renal signs and symptoms may in the early stages be slight or absent; whereas a careful study of the advanced cases may throw some light on our problems of cardiovascular pathology. Several months ago Dr. Russell, of the London Hospital, England, told me of a case of basophilic adenoma of the anterior lobe of the pituitary showing the histological changes in the kidney characteristic of malignant nephrosclerosis. It does seem more than a mere coincidence that this relatively rare vascular lesion should recur so often in the advanced cases of pituitary basophilism.

PULMONARY EMBOLISM FOLLOWING TRAUMA. J. S. McCartney, Minneapolis, Minn.

Abstract. A study of the protocols of 9882 postmortem examinations done during 6½ years reveals that in 1604 instances, or 16.2 per cent, death followed injury. In the traumatic group embolisms were found in 61 cases (3.8 per cent), and in the non-traumatic group in 222 cases (2.6 per cent). Statistically this appears to be a significant difference. The age distribution of cases was the same in the two groups. There were 6283 males and 3598 females. The incidence of embolism was 2.4 per cent in the males and 3.5 per cent in the females. There is a significant difference in the two sexes in the incidence of embolism. The greater incidence in females is not to be accounted for by the embolisms that followed childbirth.

The incidence of embolism after trauma was 2.6 per cent in the male, and 7.7 per cent in the female. This is a significant difference.

Of the 61 traumatic cases 45, or 73.7 per cent, were between the ages of 50 and 79 years. In the non-traumatic group but 59 per cent occurred between these ages.

The incidence of embolism after skull fracture was 0.2 per cent, after fracture of the ribs 0.9 per cent, after fracture of the spine 1.4 per cent, after fracture of the upper extremity 4.5 per cent, after fracture of the pelvis 3.2 per cent, after multiple fractures 4.8 per cent, after fracture of tibia and (or) fibula 14.8 per cent, and after fracture or dislocation of the femur 25 per cent. In the group of miscellaneous traumas there was an incidence of 2.2 per cent. No embolisms followed gunshot wounds. These percentages indicate the great importance of rest in the etiology of embolism, since fractures of the lower extremity require much more prolonged immobilization than other fractures. The part which operative procedures played in this series is indeterminate, although 14 were operated upon for the trauma sustained.

Before 50 years of age the maximum incidence, according to the number of cases, was only 2.9 per cent. Between 50 and 79 years of age the incidence was 7.2 to 7.3 per cent, and from 80 to 89 years of age 4.8 per cent. The very great importance of age in the causation of embolism is clearly demonstrated.

Of the 61 embolisms, 53 followed fractures or dislocation and of these 53 embolisms 28 were fractures or dislocations of the femur and 7 fractures of the tibia and (or) fibula. Only 4 of the 53 fractures were compound.

The times of occurrence of pulmonary embolism after trauma were: 1st week 9 cases, 2nd week 21, 3rd week 10, 4th week 5, and after the 4th week 16. Embolism following trauma takes place later than after operation.

Discussion

(Major V. H. Cornell, Washington.) I should like to ask if you were using a frame for the suspension of the lower extremities in the treatment of fracture, or was the leg completely immobilized?

(Dr. Thomas H. Belt, Toronto.) Dr. McCartney's interesting paper touches upon a subject to which we have been devoting considerable attention here in Toronto, namely, the autopsy incidence of pulmonary embolism. It brings out the striking frequency of embolism following trauma. We have found here, since we have made a special search for emboli in the lungs, that they occur in a large number of cases that ordinarily might be passed over if the search was

not carefully carried out. I refer particularly to the smaller types of emboli that cause infarcts of the lung, and which I believe Dr. McCartney has excluded from his series. We find that about 10 per cent of our routine autopsies show emboli that are demonstrable to the naked eye, and a large proportion of these cases have cardiac incompetence; many of them actually died from heart disease. There are many factors in the causation of thrombosis and embolism which we have to consider, and the most important etiological factor in our series seems to be slowing of the circulation.

(Dr. McCartney.) In response to Major Cornell, I cannot answer the question as to immobilization. I have not studied as yet the hospital records from that standpoint.

I might add in connection with what Dr. Belt had to say, that, in the non-traumatic cases, cardiac lesions of one type or another stand out prominently, but this is not so true in the traumatic cases.

ANATOMICAL AND EXPERIMENTAL OBSERVATIONS ON AIR EMBOLISM. W. H. Chase (by invitation), Montreal, Canada.

Abstract. The autopsy findings are described in a case of air embolism which was rapidly fatal following the incision of a large branch of the right pulmonary vein during a lobectomy operation. On opening the cranial cavity first there was found a diffuse subarachnoid hemorrhage over the tributaries of the longitudinal sinus and extending around penetrating cortical veins. Intracranial air emboli were confined to branches of middle and anterior cerebral arteries. The left heart and coronary arteries were also filled with air, but there were no perivascular hemorrhages.

In order to obtain a visual conception of living events in air embolism measured quantities of room air were injected into the aortic arch in a series of 12 rabbits, through a long cannula inserted through an opening in the right carotid artery. The observations were made possible by the adoption of an apparatus devised by Ricker and his co-workers for the study of the rabbit's mesentery *in vivo*, while it was bathed in Ringer's fluid at a constant temperature. This apparatus was particularly applicable because (1) tissue movements could constantly be observed microscopically through a water immersion lens with direct illumination, and (2), with practice, the operator could perfect a technique that permitted him to start with a vascular mesenteric bed that was almost a physiological preparation.

The experimental observations may be summarized as representing two distinct vascular effects. The first is traumatic and probably neurovascular, and the second is mechanical. (1) The traumatic neurovascular effect is independent of the nature of the irritant. It produces an immediate transient vasoconstriction of muscular arteries and terminal segments, during which the slow peripheral blood movement (prestatic state) is associated with slight diapedesis. As fatigue of muscular arteries translates vasoconstriction to vasodilatation there develops a progressive arterial hyperemia. (2) The mechanical vascular effect is due to the specific agent which is the air obstructing small arteries and arterioles. It also produces peripheral prestasis which is of rapid onset and sometimes prolonged. During this interval there occurs a more or less abundant diapedesis of red cells from the capillaries and venous side of terminal segments. This ends in either complete cessation of blood movement (stasis) or in diffusion of air through the arterioles with a return of the hyperemic state.

In both the autopsied case and in the animal experiments air emboli were confined to arteries and arterioles, while hemorrhages were confined to terminal vascular segments and small veins. Although we must accept with caution a comparison of tissue movements in animal experiments to human pathological processes, the similarity of the vascular changes in the rabbit's mesentery to those in the meningeal vessels of the autopsied case, under stimulus of the same irritant, suggests a similar neurovascular mechanism concerned in their production.

Discussion

(Dr. Esmond R. Long, Philadelphia.) I am wondering whether Dr. Chase has given some thought to that very distressing accident which occurs now and then, sudden death during the course of artificial pneumothorax. As you know, sometimes in the course of the therapeutic administration of air in a pleural cavity the patient goes into a state of collapse and dies within a few minutes. There have been two theories as to the cause of death. One is that death is due to introduction of the needle into the pulmonary vein with resultant direct air embolism. This theory has not been substantiated by postmortem examinations. In its failure a second theory has been accepted by a good many clinicians, the rather nebulous hypothesis of pleural shock. In the first case reported by Dr. Chase it is possible that a vein was punctured. If so, after the needle was withdrawn, could a sufficient amount of air have entered the pulmonary vein from the pleural cavity to cause embolism? Sometimes death occurs after 200 to 300 cc. have been put in the cavity. There is no question in these cases that during the administration of air the needle is not in the vein; the manometer shows it to be in the pleural cavity.

(Dr. Timothy Leary, Boston.) Medicolegal deaths from air embolism are rather rare, but sudden death is always due to a massive introduction of air into the circulation, making a mixture that cannot be pumped. One finds the right side of the heart filled with this frothy blood constantly in my experience. I have seen at least 10 or 15 of these cases. I have performed autopsies upon 2 cases of sudden death from pleural shock following the introduction of a needle into the lung for the purpose of obtaining lung cultures in pneumonia. There was no possibility of the introduction of air in these cases.

(Dr. Long.) I am glad to hear Dr. Leary's comment, because in Chicago we always taught that death in air embolism could occur only in the way he describes. However, it is commonly held by clinicians who do pneumothorax work that fatal embolism can take place from small amounts of air on the other side of the vascular circulation.

(Dr. Edwin F. Hirsch, Chicago.) I should like to ask Dr. Chase whether he considers that the experiments done on the peripheral circulation apply to the pulmonary circulation. There has been doubt that air introduced into the pulmonary artery passed through the capillary bed of the lungs and entered the systemic circulation.

(Dr. Marcus W. Lyon, South Bend.) I should like to cite one instance of accidental administration of air into the venous circulation. In an attempt to withdraw blood from a patient for immunization against influenza in 1918–19 the patient complained as the pump was being worked that he could feel something trickling up his arm, and the person operating it discovered that the pump was set in the wrong position, and instead of withdrawing blood, air was being

forced into the patient. Nothing happened, but the patient was sent back to the ward without further attempt at withdrawal of blood. Probably not much air entered, but certainly some.

(Dr. Harry C. Schmeisser, Memphis.) Dr. Chase's observations are very important in answer to the question so frequently put to the pathologist by the surgeon, whether or not air introduced into the circulation at operation could have a bad effect upon the patient and possibly be the cause of sudden death. In past years Dr. Welch taught that no bad effects would be experienced from air emboli except possibly, on operating about the neck, the internal jugular might be cut and a large amount of air pass to the pulmonary artery.

(Dr. Chase.) I think there is no doubt that it takes large quantities of air in the circulation to cause death in humans. We have had several cases of death from pleural shock, in none of which have we been able to demonstrate definitely the presence of air embolism. Several such cases have been described in the literature.

In regard to the passage of air through the pulmonary circulation, that does occur in some animals. It occurs in dogs, but there is nothing to indicate that it occurs in rabbits or in humans.

STUDIES ON THE DIGITAL VASCULAR SYSTEM WITH CONSIDERATION OF THE CONDITION OF THE GLOMUS IN INFLAMMATION, ARTERIOSCLEROTIC GANGRENE, DIABETIC GANGRENE AND THROMBOANGEITIS OBLITERANS. N. W. Popoff, Rochester, N. Y.

Abstract. A brief summary of the work done on the human digital vascular system is presented. A digital glomus similar to glomus coccygeum is described. Anatomical data enabling understanding of heat regulation and other functions of the digital glomus are given. The importance of taking into consideration the state of the glomus for proper understanding of capillary reading, of local clinical manifestations and of various diagnostic tests is stressed. New histological points in differentiating arteriosclerotic and diabetic gangrene of the extremities are presented. A hitherto unknown vascular anomaly manifested by the presence of peripheral digital arteriovenous anastomoses is described and is offered as an etiological factor of thromboangeitis obliterans.

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